LEADING ARTICLE

Drugs 2012; 72 (4): e1-e16
0012-6667/12/0004-0001

Nicotine Vaccines to Assist with Smoking Cessation

Current Status of Research

Tobias Raupach,[1] *Philippe H.J. Hoogsteder*[2] and *Constant P. (Onno) van Schayck*[2]

1 Department of Cardiology and Pneumology, University Hospital Göttingen, Göttingen, Germany
2 Department of General Practice, Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, the Netherlands

Abstract

Tobacco smoking causes cardiovascular, respiratory and malignant disease, and stopping smoking is among the key medical interventions to lower the worldwide burden of these disorders. However, the addictive properties of cigarette smoking, including nicotine inhalation, render most quit attempts unsuccessful. Recommended therapies, including combinations of counselling and medication, produce long-term continuous abstinence rates of no more than 30%. Thus, more effective treatment options are needed.

An intriguing novel therapeutic concept is vaccination against nicotine. The basic principle of this approach is that, after entering the systemic circulation, a substantial proportion of nicotine can be bound by antibodies. Once bound to antibodies, nicotine is no longer able to cross the blood-brain barrier. As a consequence, the rewarding effects of nicotine are diminished, and relapse to smoking is less likely to occur. Animal studies indicate that antibodies profoundly change the pharmacokinetics of the drug and can interfere with nicotine self-administration and impact on the severity of withdrawal symptoms. To date, five phase I/II clinical trials using vaccines against nicotine have been published. Results have been disappointing in that an increase in quit rates was only observed in small groups of smokers displaying particularly high antibody titres.

The failure of encouraging preclinical data to completely translate to clinical studies may be partially explained by shortcomings of animal models of addiction and an incomplete understanding of the complex physiological and behavioural processes contributing to tobacco addiction. This review summarizes the current status of research and suggests some directions for the future development of vaccines against nicotine. Ideally, these vaccines could one day become part of a multifaceted approach to treating tobacco addiction that includes counselling and pharmacotherapy.

1. Introduction

There are currently 1 billion tobacco smokers in the world,[1] at least half of whom will eventually die from a smoking-related disease.[2] By causing cardiovascular, respiratory and malignant disease, smoking accounts for 10% of global mortality.[3] Due to its pharmacokinetic properties, nicotine that is inhaled from cigarette smoke readily evokes addiction in many smokers (see Benowitz[4] for

details). Accordingly, tobacco dependence has been called a chronic disease necessitating specific treatment.[5] The treatment of tobacco use usually involves some form of counselling[6] or pharmacotherapy;[7] ideally, both approaches should be combined.[8] Currently available pharmacotherapy increases quit rates by reducing craving or providing relief from withdrawal symptoms.

Recent research regarding drugs to support quit attempts has focussed on combination therapies,[9] pharmacogenetics[10] and modifications to existing dosing regimens.[11,12] However, long-term continuous abstinence rates achieved by approved treatment options, combined with intensive counselling, rarely exceed 30%,[8] and patient adherence to treatment is modest.[13] As smokers display diverse smoking patterns[14] and different needs when trying to quit, increased diversity of treatment options would be desirable.[15] An intriguing novel concept is nicotine vaccination. The basic principle of this approach is that, after entering the systemic circulation, a substantial proportion of nicotine can be bound by antibodies. Once bound to antibodies, nicotine is no longer able to cross the blood-brain barrier. As a consequence, the rewarding effects of nicotine are diminished, and relapse to smoking is less likely to occur.[16] Preclinical experiments have yielded promising results, and, to date, five reports of phase I–II trials using nicotine vaccines in humans have been published.[17-21]

Starting from a summary of neural mechanisms involved in nicotine addiction, this review illustrates some practical aspects relevant to the design of a nicotine vaccine. An overview of principle findings from animal studies is followed by a more detailed description of clinical trial results. The article concludes with a critical appraisal of this novel therapeutic approach and some suggestions for future research.

2. Mechanisms of Nicotine Addiction

2.1 Nicotine Pharmacokinetics

Nicotine is part of the particulate phase of tobacco smoke and constitutes the most prevalent alkaloid in tobacco. It is a small molecule (162 daltons). As indicated by its chemical name, (S)-3-(1-Methylpyrrolidin-2-yl)pyridine, this natural insecticide contains a pyridine and a pyrrolidine ring, both of which are carrying a tertiary amine. The two rings possess different acid dissociation constants, producing a net acid dissociation constant (pK_a) of 8.0–8.5 (for overview, please see Hukkanen et al.[22]). The acidity of cigarette smoke (pH = 5.5–6.0) prevents large doses of nicotine from being absorbed by the oral mucosa. However, in the more alkaline environment of the pulmonary alveoli (pH 7.4), one in three nicotine molecules is non-ionized and thus capable of crossing biological membranes. The average nicotine content of a cigarette is 10–14 mg,[23] only 10% of which enters the systemic circulation,[24] resulting in peak plasma concentrations of 300–500 nM.[25] Nicotine intake from one cigarette is approximately 0.015 mg per kg,[24] and this dose has been used in most preclinical studies using nicotine infusions in animals.

The amount of nicotine binding to plasma proteins is negligible (5%[26]); its half-life is 2 hours,[27] and the greater part of nicotine is metabolized to cotinine. This conversion is almost exclusively catalyzed by one specific cytochrome P450 (CYP) isoenzyme (CYP2A6).[28] The rate of nicotine metabolism is determined by genetic[29] and hormonal[30] factors as well as concomitant medication and appears to have a direct impact on the severity of withdrawal symptoms and, ultimately, the success of a quit attempt.[31,32]

2.2 Nicotine Effects on the Brain

While most pathophysiological mechanisms underlying smoking-related disease are activated by smoke constituents other than nicotine, it has been known for several decades that nicotine effects on the brain following smoke inhalation are central to the addictive properties of smoking.[33] In the broadest sense, nicotine impacts on locomotion, cognition, affect, pain sensitivity[34] and arousal.[35] Most importantly, however, nicotine increases the brain's responsiveness to rewarding stimuli,[36] ultimately leading to addiction. Nicotine delivery kinetics to the brain appear to be crucial in facilitating this effect in that a rapid

increase in nicotine levels is much more likely to promote self-administration (a laboratory paradigm for addiction) than slow kinetics.[37,38] In humans, high levels of nicotine reach the brain within 10–20 seconds after taking a puff from a cigarette.[39] In fact, most smokers report that the subjective effects of cigarette smoking are maximal in the first few minutes after smoke inhalation.[40] This has led to the assumption that nicotine effects on the brain are primarily determined by the speed at which the compound enters the brain.[41]

Various *in vivo* and *in vitro* studies performed in the mid-1980s have identified the mesocorticolimbic dopamine system as the primary site of action regarding nicotine's reinforcing and rewarding effects.[42] This complex neuronal network involves dopaminergic neurons located in the ventral tegmental area (VTA) projecting to diverse brain regions, including the nucleus accumbens (synonyms: NAcc, ventral striatum[43]). Nicotine activates nicotinic acetylcholine receptors (nAChR)[44,45] on VTA neurons, ultimately leading to dopamine release in the NAcc.[46] Increased dopamine levels in this region generally promote the development of drug dependence.[47,48] Notwithstanding the need for a better understanding of the complex interactions of neuronal pathways leading to addiction, reward from smoking is likely to occur as a consequence of dopamine release in the NAcc following a *rapid* increase in brain nicotine concentration.

In addition to triggering dopamine release, nicotine induces desensitization of nAChRs and an increase in receptor density.[49] Receptor upregulation implies the possibility of developing a hypercholinergic state when nicotine supply is interrupted. In fact, regular smokers display an almost complete receptor saturation,[50] which may serve to prevent the occurrence of withdrawal symptoms.

2.3 Nicotine Withdrawal and Relapse to Smoking

Considering the pathophysiological basis of nicotine addiction outlined in section 2.2, the rewarding effects of peaking nicotine levels in the brain would appear to be the main drivers of nicotine addiction. However, animal research suggests that the reinforcing potential of nicotine is only moderate compared with other drugs of abuse.[51] As a consequence, pending nicotine withdrawal has been suggested to underlie continued smoking behaviour. Withdrawal can be defined as a syndrome of behavioural, affective, cognitive and physiological symptoms,[52] including depressed mood, anxiety, irritability and craving.[53] Animal research shows that spontaneous nicotine withdrawal results in a significant decrease in brain reward function.[51] Conversely, dependent smokers may use nicotine inhalations as a self-medication to relieve negative affect and other withdrawal symptoms.[54] However, there is no uniform clinical picture of withdrawal:[55] in one study,[56] three patient clusters with distinct characteristics of the syndrome were identified, highlighting the difficulties associated with developing treatments to relieve withdrawal.

Symptoms of nicotine withdrawal may act as triggers causing a smoking lapse during a quit attempt. However, other trigger factors have also been reported. Since smoking is a conditioned behaviour,[57] cues associated with smoking can urge patients to abandon their plans of quitting.[4] Likewise, consumption of coffee and alcohol,[58] specific social situations, negative feelings and times of being alone are all associated with an increased risk of experiencing a smoking lapse.[59] Particularly early lapses (occurring within the first 2 weeks of a quit attempt) are associated with low long-term success rates.[60] Progression from lapse to full-blown relapse only occurs if smoking is perceived as rewarding. Thus, further developments in pharmacotherapy for smoking cessation should focus on reducing the rewarding effects of smoking.

3. Vaccine Development: Rationale and Practical Aspects

3.1 Working Mechanism of a Nicotine Vaccine

Based on the assumptions that a rapid increase in brain nicotine levels induces feelings of reward and that quit attempts frequently fail due to early

lapses, preventing nicotine from entering the brain constitutes an intriguing concept. The idea of using antibodies to bind a drug and thus disabling it from crossing the blood-brain barrier was first tested in an animal model of heroin addiction[61,62] and subsequently extended to other species and other drugs of abuse, including morphine, methamphetamine, phencyclidine,[63] cocaine[64] and nicotine.[65] To date, only nicotine and cocaine have been used as vaccination targets in human studies.[63] The term 'vaccination' (synonym: active immunization) refers to the administration of an immunogenic substrate that causes T and B cell activation, leading to the formation of specific antibodies within the studied individual. By virtue of imprinting this response to the immunological memory, this approach yields longer-lasting protection. However, therapeutic antibody levels are only established several weeks after the first vaccine injection. Passive immunization is defined as the administration of preformed monoclonal or polyclonal high-affinity antibodies. This strategy offers immediate protection. However, in addition to being more expensive owing to high manufacturing costs, its effects are more short lived due to rapid degradation of antibodies.[66] Moreover, the amount of injectable antibodies is limited.[67] Studies in animals have used both approaches, while clinical trials have focused on active immunization procedures.

3.2 Characteristics of an Immune Response

The minimum molecular weight for a molecule to elicit a specific immune response is 10 kDa.[67] Due to its small molecular weight, nicotine itself is not immunogenic; in order to trigger an immune response, nicotine or a structurally similar hapten needs to be linked to a carrier protein,[68,69] thus producing a conjugate vaccine. The success of an immunological strategy relies on several characteristics, including but not limited to (i) immunogenicity of the vaccine; (ii) affinity of antibodies; and (iii) specificity of antibodies.

3.2.1 Immunogenicity

Immunogenicity is assessed by the antibody titre elicited by a vaccine. Titres (as measured by ELISA) of >1 : 10.000 indicate satisfactory immunogenicity.[70] Unfortunately, published reports of vaccination studies have used different measures of immunogenicity (ELISA titres,[17] geometric mean titres,[18] absolute antibody concentrations,[71] molar antibody concentrations,[19] ratio of specific and total IgG concentrations[72]), thus complicating the comparison of results between studies. In general, immunogenicity is influenced by the choice of the carrier protein and adjuvants added to the vaccine.

3.2.2 Affinity

The primary measure of antibody affinity to the target drug is the equilibrium dissociation constant K_d, i.e. the ratio of unbound to bound molecules at given concentrations.[63] High K_d values indicate low affinity as large amounts of unbound molecules are needed to induce formation of complexes between nicotine and its antibodies. High affinity is desired in order to ascertain fast clearance of unbound nicotine from the plasma; however, extremely high affinity may be disadvantageous as saturation of all antibodies can be achieved after one single nicotine dose, thus compromising efficacy for subsequent nicotine doses.[73] Total binding capacity of the serum is influenced by both immunogenicity and affinity.[63,74] Thus, lower affinity can be compensated for by increasing antibody dose.[73]

3.2.3 Specificity

This term refers to the extent to which the elicited antibodies bind to nicotine with high specificity in preference to other molecules, indicating low cross-reactivity between the antibody and nicotine metabolites such as cotinine, nicotine-N-oxide and nornicotine.[74] High specificity is particularly important if these metabolites occur in higher concentrations than the drug itself, as is the case for cotinine. Cross-reactivity can be reduced by exposing nicotine's pyrrolidine ring to the immune system as this ring is first metabolized and thus not contained in most nicotine derivatives.[74] At the same time, cross reactivity might be desired if drug metabolites are themselves pharmacologically active, as is the case for cotinine[75-77] and nornicotine.[78] This

has practical implications for the design of conjugate vaccines. For example, one recent study showed that using longer rather than shorter linkers and attaching them to the 6- rather than the 5-position of the pyridine ring (i.e. further away from the prime site of nicotine metabolism[79]) increases antibody selectivity for nicotine.[74]

4. Preclinical Studies

4.1 Objectives of Animal Studies

Antibodies binding a drug will primarily change its pharmacokinetic profile[63] which, in the case of nicotine, should abolish the functionally critical brain concentration peak, making nicotine intake less rewarding.[66] Accordingly, the primary objectives of preclinical studies were to study the effects of nicotine antibodies on (i) pharmacokinetics (serum and brain nicotine concentrations following acute and chronic administration of the drug; nicotine half-life in serum); (ii) brain function and behaviour (dopamine release in the NAcc, nicotine self-administration); and (iii) signs of withdrawal.

4.2 Methodology

A comprehensive description of the experimental procedures used to model addiction in animals is beyond the scope of this review (see LeSage et al.[66] for details). Both passive and active immunization approaches have been used to study the effects of nicotine antibodies in animals, and the vast majority of research was done on rats. Passive immunization was usually performed by intravenous[80] or (rarely) intraperitoneal[81] application of antibodies. Most studies used the nicotine-specific monoclonal antibody Nic311 with a K_d value of 60 nM.[73] Vaccination involved the singular or repeated intraperitoneal administration of an immunogen;[70,71,82] however, subcutaneous and intranasal vaccine delivery has also been reported.[83] The majority of vaccination studies were done with one of the following:

- 6-(carboxymethylureido)-(6)-nicotine (CMUNic) linked to keyhole limpet hemocyanin (KLH)[84]
- 3-aminomethyl-nicotine (3′-AmNic) linked to *Pseudomonas aeruginosa* exoprotein A (rEPA)[65]
- the nicotine derivate IP18 linked to KLH[85]
- nicotine coupled to virus-like particles formed by the coat protein of the bacteriophage Qb.[19]

In order to produce a satisfactory immune response, these vaccines need to be combined with an adjuvant. The synthesis of a further immunogenic compound has been reported in the literature. This peptide-based vaccine containing a residue of an agonist of human complement factor C5a does not require the simultaneous administration of an adjuvant. To date, only one experimental study using this vaccine has been published.[86]

4.3 Principal Findings

This section describes principal findings from animal studies of passive immunization and vaccination against nicotine (table I). Following a brief discussion of antibody properties reported in the literature, the three primary objectives mentioned in section 4.1 will be addressed.

4.3.1 Antibody Characteristics Following Vaccination

Following repeated vaccination with CMUNic-KLH[84,88] or 3′-AmNic-rEPA,[65,82] antibody titres as determined by ELISA were greater than 1 : 10 000, and antibody concentration ranged from 100 μg/mL[84] to 300 μg/mL.[71] Four injections of 3′-Am-Nic-rEPA elicited titres between 1 : 200 000 and 1 : 250 000,[70] comparable to those produced by two injections of IP18-KLH.[96] In one experiment, antibody concentrations following simultaneous bivalent vaccination with 3′-AmNic-rEPA and 6-CMUNic-KLH were greater than those elicited by monovalent vaccination.[98] Notably, antibody formation did not appear to be impeded by the presence of nicotine during the vaccination procedure.[82]

The dissociation constant K_d of antibodies was within the range of 10–30 nM,[98] and nicotine binding capacity was approximately 2×10^{-6} M (equivalent to 210 ± 110 ng/mL). Nicotine-specific IgG represented approximately 1–2% of total IgG.[65,84,88]

Antibodies yielded high specificity for nicotine,[65,85,87] with only minor cross-reactivity to major metabolites of the drug.[19,74,83]

Table I. Effects of passive immunization and vaccination against nicotine on pharmacokinetics, brain function and behaviour and withdrawal in animal studies

Parameter	Passive immunization	Vaccination
Pharmacokinetics		
Serum/plasma nicotine concentration following acute nicotine administration	Increased 6- to 18-fold[65]	Increased 6-[84,87] to 8.5-fold[65]
Serum/plasma nicotine concentration following chronic nicotine administration	Increased 6-fold[65]	Increased 2- to 4-fold[88]
Brain nicotine concentration following acute nicotine administration	Reduced by 30%,[65] 40%,[80] 80%[73] or 90%[80]	Unchanged[84] or reduced by 35–40%,[87] 50–60%[65,82,89,90] or 90%[71,83]
Brain nicotine concentration following chronic nicotine administration	Unchanged[91] or reduced by 13%[65]	Reduced by 30%[71,82,88,89]
Serum nicotine half-life	Increased 2.2-fold[73]	Increased 8-fold[88]
Brain function and behaviour		
Increase of dopamine release in the nucleus accumbens following acute nicotine administration		Abolished[85]
Increase in locomotor activity following acute nicotine administration	Attenuated[65,92]	Attenuated[92]
Nicotine discrimination	Decreased[93]	
Acquisition of nicotine self-administration		Reduced (not significant)[70]
Maintenance of nicotine self-administration		Reduced[70]
Nicotine intake in a self-administration setting		Increased[94]
Reinstatement of nicotine self-administration following extinction		Abolished[95]
Withdrawal		
Withdrawal signs in the presence of nicotine	Unchanged[80]	Unchanged[96]
Withdrawal signs in the absence of nicotine	Reduced[97]	Reduced[96]
Reduction of withdrawal signs following acute nicotine administration	Attenuated[81]	Reduced[96]

4.3.2 Pharmacokinetics

Binding of nicotine to antibodies is expected to result in greater retention of the drug in the serum. Accordingly, serum nicotine concentrations following acute administration were increased at least 6-fold in both passively immunized and vaccinated rats as compared with controls.[65] The increase was slightly smaller in vaccinated rats receiving five nicotine injections over a period of 80 minutes, simulating repeated exposure as present in smokers.[88] Despite increased total nicotine levels, the fraction of unbound (and thus active) drug was reduced by up to 92%[89] as evidenced by attenuated cardiovascular effects in passively immunized animals[65] and the absence of seizures in vaccinated rats that would normally be observed following high-dose nicotine infusion.[99] Since nicotine binding partially protects the drug from being metabolized, plasma half-life of nicotine was increased 2.2- to 8-fold by passive immunization[73] and vaccination,[88] respectively.

As outlined in previous sections, the primary aim of binding nicotine in the serum is to prevent the drug from entering the brain. In most preclinical experiments, brain nicotine concentration was measured shortly (i.e. 3 minutes) after a nicotine bolus injection. Results varied significantly between studies: following acute administration, passive immunization decreased brain levels of nicotine by 30%[65] to 90%[80] compared with controls; similarly, variable reductions were observed in vaccinated rats[65,71,82,83,87,89,90] receiving a single nicotine bolus. In vaccinated rats, reductions in brain nicotine concentration were greatest shortly after acute administration of nicotine.[89] Interestingly, regardless of the mode of immunization, reductions in brain nicotine levels were considerably smaller following the chronic administration of nicotine.[65,71,82,88,89,91]

Effects of immunization on nicotine pharmacokinetics were dose dependent in that higher antibody concentrations elicited more profound nicotine retention in serum and a larger decrease in brain nicotine concentration.[65,73,80]

4.3.3 Brain Function and Behaviour

An acute increase of dopamine output in the NAcc following nicotine administration is believed to mediate the addictive properties of smoking. In vaccinated rats, this increase was abolished.[85] Likewise, the increase in locomotor activity following acute nicotine exposure was attenuated in both vaccinated[92] and passively immunized[65] animals, and the combination of both approaches further increased this effect.[100,101] Moreover, in the presence of antibodies, rats were less able to discriminate nicotine from saline injections.[93] The effect of vaccination on the development and maintenance of nicotine addiction was studied in experiments using rats that were trained to self-administer nicotine[102] at clinically relevant doses (0.01–0.06 mg/kg per dose, eliciting a $[nicotine]_{plasma}$ of 10–60 ng/mL[103]). While the acquisition of nicotine self-administration was not significantly impeded by the vaccine, maintenance of this learned behaviour was attenuated. One study found no evidence of compensatory overdosing aimed at surmounting the effects of vaccination,[70] while the results of another more recent study reported increased nicotine intake following vaccination. This might have been due to lower antibody concentrations and higher doses of nicotine infusions in the latter study, resulting in saturation of antibody binding sites and, consequently, insufficient sequestering of the drug.[94]

Even more importantly, vaccination abolished the reinstatement of nicotine self-administration following extinction of the conditioned response and re-exposure to nicotine, simulating a smoking lapse.[95] This effect was dependent on high antibody levels and was more pronounced with smaller priming doses of nicotine, resembling intake from one-tenth of a cigarette.[95]

4.3.4 Withdrawal

Due to a lack of effect on brain nAChRs, immunization against nicotine is unlikely to modulate withdrawal symptoms. On the contrary, it may be hypothesized that immunization during smoking might induce withdrawal as newly formed antibodies abolish brain nicotine peaks, which are essential for feelings of reward to occur. Assessment of nicotine withdrawal symptoms in an animal model of addiction is difficult. However, the available data suggest that neither passive immunization[80] nor vaccination[96] precipitate withdrawal in the presence of nicotine. Moreover, withdrawal following the termination of nicotine administration was reduced in immunized rats as compared with controls,[96] although there was evidence of persisting nicotine dependence.[97] Finally, one study investigated the effects of passive immunization on the reduction of withdrawal signs following re-exposure to nicotine.[81] In the presence of antibodies against nicotine, withdrawal relief achieved by nicotine administration was attenuated. Similar findings were reported following vaccination.[96]

4.4 Summary of Findings from Animal Studies

Vaccination of rats produced antibodies with high affinity and specificity for nicotine. One encouraging finding regarding the possibility of vaccinating active smokers was that immunogenicity of the vaccine was not impaired by the presence of nicotine. Animal studies have confirmed the hypothesis that nicotine pharmacokinetics are markedly changed in the presence of antibodies against nicotine. Effects on serum and brain concentrations of the drug were dependent on antibody concentration, greatest shortly after the application of a single nicotine dose and more pronounced when smaller priming doses were used. While vaccination may prevent acute nicotine delivery to the brain, chronic accumulation of the drug in the brain was only moderately affected by the presence of antibodies, i.e. nicotine entry into the brain was delayed rather than fully prevented. Accordingly, acute nicotine effects in dopamine release in the NAcc and related behaviours were attenuated in vaccinated animals. However, vaccination did not interfere with the development of nicotine addiction in a self-administration paradigm, and, even with favourable antibody titres,

maintenance of nicotine self-administration was not affected by vaccination in some animals.[70] The attenuation of withdrawal severity observed in vaccinated animals might be explained by prolonged nicotine half-life in the presence of antibodies. The finding of reduced alleviation of withdrawal symptoms by a priming dose of nicotine in vaccinated rats suggests that nicotine vaccination might be most effective in preventing relapse in smokers trying to quit.[66,67,104]

5. Clinical Studies

At least four vaccines against nicotine are currently under clinical development,[74] but peer-reviewed reports have only been published for two of them:

1. NicVAX[18,20,21] contains 3′-AmNic linked to rEPA. This vaccine was developed by Nabi Pharmaceuticals and is currently being further evaluated for clinical use by GlaxoSmithKline.
2. NIC002 (synonym: NicotineQb[17,19]) contains a nicotine derivative coupled to a virus-like particle formed by the coat protein of the bacteriophage Qb in *Escherichia coli*. This vaccine was developed by Cytos Biotechnology; further clinical evaluation is being carried out by Novartis.

All published clinical trials used alum as an adjuvant to boost immune responses. Additional vaccines currently being tested are TA-NIC (containing recombinant cholera toxin B; developed by Celtic Pharma/Xenova[105]) and Niccine (developed by Pharmaceutica AB[74]), and at least two further companies are currently evaluating nicotine vaccines at the preclinical stage with no published reports available at the time of data collection for this review. A summary of preliminary results published on company websites can be found elsewhere.[106]

Results of the five published reports of phase I/II studies are summarized in table II. In the following section, principal results regarding safety, immunogenicity and efficacy are reviewed.

5.1 Study Design

Three studies (two using NicVAX[18,20] and one using NIC002[19]) were primarily designed to assess safety and immunogenicity and therefore included smaller groups of non-smokers or smokers not intending to quit; cessation counselling was not offered in these studies. Two more recent trials also assessing efficacy included smokers willing to quit.[17,21] In these studies, subjects were required to set a quit date following their second vaccination, and cessation counselling was provided. The comparability of results is impaired by the fact that counselling was only provided in two out of five trials. Studies differed regarding vaccine doses used and vaccination schemes (for details see table II).

5.2 Safety

Mild to moderate local reactions at injection sites (ache and tenderness) were common in all studies. Systemic reactions, which were also mild in most cases, included myalgia, malaise and headaches. The prevalence of these symptoms was similar in participants receiving a vaccine and those receiving adjuvant without the active vaccine, indicating that adverse events were not related to the vaccine itself.[20,21] Serious adverse events potentially related to treatment were rarely observed: one subject with a history of allergy to penicillin was reported to have suffered from an anaphylactic reaction 70 minutes after receiving the first 400 μg dose of NicVAX.[21]

5.3 Immunogenicity

The immune response to nicotine vaccination was highly variable in all five trials. As a common feature, higher vaccine doses elicited stronger responses, and antibody titres tended to peak following the final injection although this was not observed in all studies.[20] Comparison of immunogenicity across trials is complicated as some studies reported geometric mean titres, while others assessed ELISA titres. The maximum antibody concentration in the most recent proof-of-concept study was 45 μg/mL.[21] By including both smokers and non-smokers, one study demonstrated that immunogenicity was not impeded by the presence of nicotine.[18] Antibody affinity to nicotine was only assessed in one trial using NIC002; the K_d value was 33 nM. Data on anti-

Table II. Results of phase I/II clinical trials of vaccines against nicotine

Study (phase)	Subjects and procedures	Safety	Immunogenicity	Effects on smoking
Hatsukami et al.[20] (phase I)	• 68 smokers not intending to quit • Three doses of NicVAX (50/100/200 μg) or PL • Four injections (d 0, 28, 56, 182) • Follow-up: 80 d	• Local reactions (99.5% mild/moderate): ache 90%, tenderness 88%, burning 40%, induration 34%, heat 26%, redness 30% • Systemic reactions (89% mild): headache 74%, myalgia 71%, malaise 68%, nausea 26%, fever 20% • No differences between PL and verum groups	• Wide range of antibody concentrations • Maximum antibody concentration was reached after the third injection and highest in the 200 μg group (25 μg/mL)	• No compensatory smoking following vaccination • No change in withdrawal symptoms following each injection • Significant dose effect for the number of subjects achieving 30 d abstinence ($p=0.01$)
Maurer et al.[19] (phase I)	• 40 healthy volunteers • Two doses of NIC002 (50/100 μg) or PL • Two injections 4 wk apart	• Local reactions: present in all participants • Systemic reactions: headache 34%, malaise 28%	• Responder rate 100% after the first injection • 1.5-fold increase in titres by addition of alum	
Wagena et al.[18] (phase I/II)	• 9 non-smokers, 21 smokers not intending to quit • NicVAX 100 μg or PL • Four injections (d 0, 14, 28, 182) • Follow-up: 80 d	• Local reactions (81% mild): tenderness 67%, ache 50%, swelling/induration 38% • Systemic reactions (85% mild): myalgia 38%, headache 25%, malaise 25% • No differences between PL and verum groups	• No interference of antibody formation with smoking status • Geometric mean titre was maximal (10.8 μg/mL) after the fourth injection	
Cornuz et al.[17] (phase II)	• 341 smokers willing to quit • NIC002 100 μg or PL • Five monthly injections • Cessation counselling • Follow-up: 6 mo	• Overall severity of adverse events: 96% mild to moderate; 8 serious adverse events, one of which (flu-like symptoms and chest pain) was possibly related to treatment • Local reactions: pain 20% • Systemic reactions: flu-like symptoms 69%, fever 42%, headache 40%, rigors (chills)14%, myalgia 14%	• Responder rate 100% after the first injection • Peak titres after the fifth injection	• No compensatory smoking following vaccination • ITT analysis: similar abstinence rates in verum and PL groups • PP analysis: 12 mo abstinence 42% in high responders vs 21% in PL (OR 2.6, 95% CI 1.2, 5.7; $p=0.012$).
Hatsukami et al.[21] (phase II)	• 301 smokers willing to quit • Two doses of NicVAX (200/400 μg) or PL • Four or five injections over 6 mo • Cessation counselling • Follow-up: 6 mo	• Predominantly mild to moderate adverse events; 18 serious adverse events, one of which (anaphylaxis) was possibly related to treatment • Local reactions: tenderness 95%, ache 94%, burning 44%, redness 47%, heat 46%, swelling/induration 62% • Systemic reactions: myalgia 85%, malaise 79%, headache 68%, nausea 44%, fever 10%, vomiting 7%	• Each booster dose further increased antibody levels • Maximum antibody concentration 45 μg/mL	• ITT analysis: significant difference between verum and PL only following five 400 μg injections • 12 mo abstinence 19.7% in high responders vs 6.0% in PL (OR 4.41, 95% CI 1.53, 12.71; $p=0.006$).

ITT = intention-to-treat; **OR** = odds ratio; **PL** = placebo; **PP** = per protocol.

body specificity following vaccination in humans have not been published.

5.4 Efficacy

Three trials reported data on smoking status.[17,20,21] Following vaccination, there was no evidence of compensatory smoking in order to overcome the effects of antibodies blocking nicotine entry into the brain. At the same time, vaccination did not appear to induce withdrawal symptoms in smokers.

The first study formally assessing continuous abstinence rates was published in 2008.[17] According to the intent-to-treat analysis, there was no significant difference between 3-month continuous abstinence rates until month 6 between the intervention and control groups (30.1% vs 26.1%). However, a per-protocol analysis excluding all subjects with concomitant use of nicotine replacement therapy revealed significantly higher abstinence rates in the upper third of responders (i.e. subjects with particularly high antibody titres) as compared with the placebo group (56.6% vs 31.3%; odds ratio [OR] 2.9, 95% CI 1.4, 5.9; $p = 0.004$). This difference was maintained until 12 months (41.5% vs 21.3%; OR 2.6, 95% CI 1.2, 5.7; $p = 0.012$).

Based on these results, a subsequent trial was intended to further establish a relationship between antibody titres and smoking outcome.[21] Continuous abstinence rates at 12 months were significantly higher in the top 30% responders than in subjects receiving placebo (19.7% vs 6.0%; OR 4.41, 95% CI 1.53, 12.71; $p = 0.006$). In addition, continuous abstinence was enhanced in subjects receiving the most intensive vaccination regimen. Finally, among smokers who failed to quit, smoking reduction occurred more frequently in subjects with high antibody titres ('responders') compared with placebo.

Recently NABI Biopharmaceuticals announced the results of the first phase III, double-blinded, placebo-controlled trial including 1000 subjects who received a total of six vaccinations with NicVAX 400 μg or placebo. A preliminary assessment of the trial data showed that continuous abstinence for 16 weeks, from week 37 to 52, was similar in both treatment arms and approximately 11%.[107] However, a peer-reviewed report of these results has not been published yet.

5.5 Summary of Findings from Phase I/II Trials

Taken together, published results of phase I/II studies indicate that nicotine vaccination is well tolerated and capable of inducing an immune response against nicotine even with concomitant smoking. However, in contrast to expectations from animal studies, vaccination failed to increase continuous abstinence rates over placebo.

6. Strengths and Limitations; Suggestions for Future Research

The basic idea of preventing nicotine from entering the brain, thereby blocking its rewarding effects in the mesolimbic system is attractive as antibodies are unlikely to elicit adverse effects within the brain[67] that have been described for other smoking-cessation medications.[108,109] In fact, no major adverse events following vaccination were reported in phase I/II clinical trials. A further advantage of this novel approach is that treatment adherence is likely to be favourable[110] as vaccination requires only a limited number of injections and no daily dosing. Clinical trials have partially confirmed preclinical observations (e.g. lack of compensatory smoking), but they failed to establish a clear effect of vaccination on smoking behaviour.

Knowledge of the reasons for this failure of the vaccination concept to successfully translate to the clinical setting is crucial for the future development of nicotine vaccines. One obvious limitation of phase I/II trials was that they were not designed to assess efficacy endpoints. Moreover, the proportion of subjects receiving all scheduled vaccinations was as low as 60% in one trial.[21] Choosing a target quit date shortly after the first booster injection might also have impaired efficacy, as peak antibody titres were only observed following several vaccinations. In addition, as nicotine-binding capacity is not only determined by antibody concentration, a more thorough investigation of antibody affinity and specificity in these trials would have been de-

sirable. However, some issues going beyond these practical aspects need to be addressed in order to understand the obvious discrepancy between preclinical and clinical findings.

6.1 Tobacco Addiction

Recent research into the pathophysiology of drug dependence indicates that viewing nicotine peaks and subsequent dopamine release in the NAcc as an indispensable element of tobacco addiction may be too simplistic. Thus, dopaminergic VTA neurons have been found to increase their activity not only in response to nicotine but also in the presence of aversive stimuli,[111] and animal studies suggest that hedonic responses can be elicited in the absence of dopamine.[112] The finding of increased smoking-cessation rates following damage to the insula[113] further substantiates the notion that the mesolimbic reward circuit is but one brain structure relevant to nicotine addiction. Finally, the whole concept of nicotine concentration peaks underlying the rewarding effects of smoking has been questioned by a positron emission tomography (PET) study suggesting a gradual increase in brain nicotine levels during smoking with no evidence of concentration peaks following each puff.[114]

Until recently,[115] the non-nicotinic components of tobacco addiction[116] have received relatively little scientific attention. It is now recognized that tobacco addiction results from an interplay of pharmacological, psychological, genetic and environmental factors.[4] The contribution of nicotine to this complex interaction may be smaller than previously thought[117] as rewards elicited by smoking can also be induced by smoking denicotinized cigarettes.[118] In fact, substances in smoke other than nicotine have been found to enhance the reinforcing properties of nicotine.[119] Among these, acetaldehyde[120] and agents blocking monoamine oxidase activity[121] may play a major role. Results of animal experiments indicate that nicotine and other agents might support early conditioning of the reward response triggered by smoking-related cues. At a later stage, these conditioned stimuli might be sufficient to generate feelings of reward.[122]

6.2 Limitations of Animal Models

At first glance, preclinical studies provide convincing evidence that a nicotine vaccine is likely to reduce nicotine self-administration[70] and possibly even attenuate withdrawal symptoms,[96] thus facilitating smoking cessation and preventing relapse. However, although great efforts were made to adjust nicotine concentrations in animals to those observed in smokers,[123] the extent to which these findings can be applied to tobacco addiction in humans remains unclear.

First, much of this evidence relates to serum and brain nicotine concentrations measured shortly after the intravenous application of nicotine (table I). Most animal models did not use nicotine inhalation as the primary route of drug administration. In addition, these experiments have not assessed temporal patterns of nicotine distribution to different organs including the brain[114] and the lungs.[71,89,114] The clinical significance of reducing nicotine distribution to the brain by 40–60% at one fixed time-point is unclear[82] as it is the *persistence* of nicotine in the brain that appears to modulate structure and function of nAChRs.[4]

Second, the immune response elicited by vaccination against nicotine may differ from species to species. Accordingly, antibody levels reported in rats ranged from 100 μg/mL[84] to 300 μg/mL,[71] while much lower concentrations (up to 45 μg/mL[21]) were observed in clinical trials. Yet, this difference might be attributable to the heterogeneous methodology in determining antibody levels.

Third, as the rewarding properties of nicotine are limited,[51] preclinical research paradigms focusing on reinforcement[102] may not be sufficient to model the complex pharmacological and behavioural aspects of smoking and cessation. In fact, animal studies have rarely used the cues and psychosocial processes associated with relapse.[124] Even when cues were used,[95] the validity of experimental extinction of these conditioned stimuli to mirror quit attempts in humans is highly debatable.

6.3 Clinical Trial Design

As an introduction of nicotine vaccines to international markets will depend on the results of future trials, these studies need to be carefully

designed. Despite the conclusion drawn from preclinical studies that a nicotine vaccine might be primarily useful in preventing relapse, published clinical trials were designed as smoking-cessation studies including target quit dates. They mainly assessed continuous abstinence rather than withdrawal severity, the incidence of lapses and their progression to full relapse.[125] Variables that are known to impact on continuous abstinence, including the speed of nicotine metabolism,[126-130] have not been investigated in vaccination studies. In addition, antibody characteristics, including affinity and specificity, clearly have a huge impact on functionality[74] and thus require thorough evaluation in clinical trials. Careful selection of primary study endpoints and secondary measurements is therefore warranted for these trials, and study populations must be aligned to these endpoints.

Smokers who have already quit may represent an ideal target group for a relapse prevention study. However, the formation of sufficient amounts of antibodies takes at least 6 weeks, while most relapse occurs within the first 2 weeks of a quit attempt.[131] As a consequence, subjects would have to be enrolled well before quitting, which may be difficult as 50% of quit attempts occur spontaneously.[132] Accordingly, offering nicotine vaccination to smokers willing to reduce their cigarette consumption with the ultimate goal of quitting could be a viable option. Once these smokers feel prepared to quit, adequate antibody levels might be readily available.

Finally, monotherapy with a nicotine vaccine does not appear to be a promising tool to overcome the complex physiological and psychosocial alterations observed in smokers. Thus, a nicotine vaccine with sufficient efficacy could one day become part of a multifaceted intervention[104] that includes behavioural as well as pharmacological approaches. Regarding the latter, varenicline might be used in combination with the vaccine as it produces high abstinence rates at the end of treatment,[133] after which relapse needs to be prevented. A 1-year study combining varenicline with placebo-controlled NicVAX focussing on relapse prevention in almost 600 smokers is underway and may help to unravel a possible synergism between these drugs. The utility of nicotine replacement therapy, bupropion and novel agents such as cytisine[134,135] in this setting also needs to be established. Finally, smokers who decide to quit spontaneously and seek immediate help might be offered passive immunization followed by pharmacotherapy to reduce craving and withdrawal symptoms while vaccination is started concomitantly in order to establish adequate antibody titres to prevent relapse at a later stage.

7. Conclusions

Vaccination against nicotine is an intriguing novel therapeutic approach to treating nicotine addiction.[136] By preventing nicotine from entering into the brain, antibodies might abolish the rewarding properties of smoking. Animal studies indicate that antibodies profoundly change the pharmacokinetics of the drug and can interfere with nicotine self-administration. However, in recent phase I/II trials and one unpublished phase III trial, these findings were not fully replicated. In addition to assessing alternative routes of vaccine administration[137,138] and addressing ethical issues generally associated with vaccinations against drugs of abuse,[139,140] future studies will need to more clearly define the ideal indications and target populations for nicotine vaccines. Treatment of dependent smokers cannot be reduced to a course of vaccine injections but needs to acknowledge the complex pharmacological and behavioural aspects of smoking.

Acknowledgements

The authors declare that they did not receive any funding for the preparation of this review.

T. Raupach has received grants to conduct investigator-initiated studies from Pfizer and Johnson & Johnson. He has been reimbursed for attendance and lecturing at several Pfizer symposia on smoking cessation from 2006 through 2011. He has also received travel expenses and honoraria from GlaxoSmithKline and Novartis. None of these activities were related to this manuscript. C.P.v. Schayck received funding for research proposals from GlaxoSmithKline and Pfizer, as well as reimbursement for lecturing from Pfizer. PHJ Hoogsteder has no conflicts of interest to declare.

References

1. WHO. WHO report on the global tobacco epidemic, 2011: warning about the dangers of tobacco. Geneva: WHO, 2011 [online]. Available from URL: http://whqlibdoc.

who.int/publications/2011/9789240687813_eng.pdf [Accessed 2012 Feb 14]

2. Doll R, Peto R, Boreham J, et al. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ 2004 Jun 26; 328 (7455): 1519
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006 Nov; 3 (11): e442
4. Benowitz NL. Nicotine addiction. N Engl J Med 2010 Jun 17; 362 (24): 2295-303
5. Steinberg MB, Schmelzer AC, Richardson DL, et al. The case for treating tobacco dependence as a chronic disease. Ann Intern Med 2008 Apr 1; 148 (7): 554-6
6. Lancaster T, Stead LF. Individual behavioural counselling for smoking cessation. Cochrane Database Syst Rev 2005 Apr 18; (2): CD001292
7. Raupach T, van Schayck CP. Pharmacotherapy for smoking cessation: current advances and research topics. CNS Drugs 2011 May 1; 25 (5): 371-82
8. Fiore MC, Jaén CR, Baker TB. Treating tobacco use and dependence: 2008 update. Clinical practice guideline. Rockville (MD): US Department of Health and Human Services; Public Health Service, 2008 May
9. Ebbert JO, Hays JT, Hurt RD. Combination pharmacotherapy for stopping smoking: what advantages does it offer? Drugs 2010 Apr 16; 70 (6): 643-50
10. Kortmann GL, Dobler CJ, Bizarro L, et al. Pharmacogenetics of smoking cessation therapy. Am J Med Genet B Neuropsychiatr Genet 2010 Jan 5; 153B (1): 17-28
11. Rennard S, Hughes J, Cincirpini PM, et al. A randomized placebo-controlled trial of varenicline for smoking cessation allowing flexible quit dates. Nicotine Tob Res. Epub 2011 Nov 11
12. Lindson N, Aveyard P. An updated meta-analysis of nicotine preloading for smoking cessation: investigating mediators of the effect. Psychopharmacology (Berl) 2011 Apr; 214 (3): 579-92
13. Balmford J, Borland R, Hammond D, et al. Adherence to and reasons for premature discontinuation from stop-smoking medications: data from the ITC Four-Country Survey. Nicotine Tob Res 2011 Feb; 13 (2): 94-102
14. Shiffman S. Light and intermittent smokers: background and perspective. Nicotine Tob Res 2009 Feb; 11 (2): 122-5
15. Buchhalter AR, Fant RV, Henningfield JE. Novel pharmacological approaches for treating tobacco dependence and withdrawal: current status. Drugs 2008; 68 (8): 1067-88
16. Vocci FJ, Chiang CN. Vaccines against nicotine: how effective are they likely to be in preventing smoking? CNS Drugs 2001; 15 (7): 505-14
17. Cornuz J, Zwahlen S, Jungi WF, et al. A vaccine against nicotine for smoking cessation: a randomized controlled trial. PLoS One 2008; 3 (6): e2547
18. Wagena EJ, de Vos A, Horwith G, et al. The immunogenicity and safety of a nicotine vaccine in smokers and nonsmokers: results of a randomized, placebo-controlled phase 1/2 trial. Nicotine Tob Res 2008 Jan; 10 (1): 213-8
19. Maurer P, Jennings GT, Willers J, et al. A therapeutic vaccine for nicotine dependence: preclinical efficacy, and phase I safety and immunogenicity. Eur J Immunol 2005 Jul; 35 (7): 2031-40
20. Hatsukami DK, Rennard S, Jorenby D, et al. Safety and immunogenicity of a nicotine conjugate vaccine in current smokers. Clin Pharmacol Ther 2005 Nov; 78 (5): 456-67
21. Hatsukami DK, Jorenby DE, Gonzales D, et al. Immunogenicity and smoking-cessation outcomes for a novel nicotine immunotherapeutic. Clin Pharmacol Ther 2011 Mar; 89 (3): 392-9
22. Hukkanen J, Jacob 3rd P, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev 2005 Mar; 57 (1): 79-115
23. Kozlowski LT, Mehta NY, Sweeney CT, et al. Filter ventilation and nicotine content of tobacco in cigarettes from Canada, the United Kingdom, and the United States. Tob Control 1998 Winter; 7 (4): 369-75
24. Benowitz NL, Jacob 3rd P. Daily intake of nicotine during cigarette smoking. Clin Pharmacol Ther 1984 Apr; 35 (4): 499-504
25. Henningfield JE, Stapleton JM, Benowitz NL, et al. Higher levels of nicotine in arterial than in venous blood after cigarette smoking. Drug Alcohol Depend 1993 Jun; 33 (1): 23-9
26. Benowitz NL, Jacob 3rd P, Jones RT, et al. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther 1982 May; 221 (2): 368-72
27. Benowitz NL, Jacob 3rd P. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clin Pharmacol Ther 1993 Mar; 53 (3): 316-23
28. Berkman CE, Park SB, Wrighton SA, et al. In vitro-in vivo correlations of human (S)-nicotine metabolism. Biochem Pharmacol 1995 Aug 8; 50 (4): 565-70
29. Benowitz NL, Swan GE, Jacob 3rd P, et al. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. Clin Pharmacol Ther 2006 Nov; 80 (5): 457-67
30. Benowitz NL, Lessov-Schlaggar CN, Swan GE, et al. Female sex and oral contraceptive use accelerate nicotine metabolism. Clin Pharmacol Ther 2006 May; 79 (5): 480-8
31. Lerman C, Tyndale R, Patterson F, et al. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. Clin Pharmacol Ther 2006 Jun; 79 (6): 600-8
32. Patterson F, Schnoll RA, Wileyto EP, et al. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. Clin Pharmacol Ther 2008 Sep; 84 (3): 320-5
33. Balfour DJ. Neural mechanisms underlying nicotine dependence. Addiction 1994 Nov; 89 (11): 1419-23
34. Picciotto MR, Caldarone BJ, King SL, et al. Nicotinic receptors in the brain: links between molecular biology and behavior. Neuropsychopharmacology 2000 May; 22 (5): 451-65
35. Rose JE, Behm FM, Westman EC, et al. PET studies of the influences of nicotine on neural systems in cigarette smokers. Am J Psychiatry 2003 Feb; 160 (2): 323-33
36. Kenny PJ, Markou A. Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. Neuropsychopharmacology 2006 Jun; 31 (6): 1203-11
37. Henningfield JE, Keenan RM. Nicotine delivery kinetics and abuse liability. J Consult Clin Psychol 1993 Oct; 61 (5): 743-50

38. Samaha AN, Yau WY, Yang P, et al. Rapid delivery of nicotine promotes behavioral sensitization and alters its neurobiological impact. Biol Psychiatry 2005 Feb 15; 57 (4): 351-60
39. Benowitz NL, Porchet H, Sheiner L, et al. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther 1988 Jul; 44 (1): 23-8
40. Henningfield JE, Miyasato K, Jasinski DR. Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. J Pharmacol Exp Ther 1985 Jul; 234 (1): 1-12
41. Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol 1996; 36: 597-613
42. Corrigall WA, Franklin KB, Coen KM, et al. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology (Berl) 1992; 107 (2-3): 285-9
43. Pierce RC, Kumaresan V. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 2006; 30 (2): 215-38
44. Dome P, Lazary J, Kalapos MP, et al. Smoking, nicotine and neuropsychiatric disorders. Neurosci Biobehav Rev 2010 Mar; 34 (3): 295-342
45. Leonard S, Bertrand D. Neuronal nicotinic receptors: from structure to function. Nicotine Tob Res 2001 Aug; 3 (3): 203-23
46. Suaud-Chagny MF, Chergui K, Chouvet G, et al. Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. Neuroscience 1992 Jul; 49 (1): 63-72
47. Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci 2005 Nov; 8 (11): 1445-9
48. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 1988 Jul; 85 (14): 5274-8
49. Picciotto MR, Addy NA, Mineur YS, et al. It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. Prog Neurobiol 2008 Apr; 84 (4): 329-42
50. Brody AL, Mandelkern MA, London ED, et al. Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. Arch Gen Psychiatry 2006 Aug; 63 (8): 907-15
51. Epping-Jordan MP, Watkins SS, Koob GF, et al. Dramatic decreases in brain reward function during nicotine withdrawal. Nature 1998 May 7; 393 (6680): 76-9
52. Shiffman S, West R, Gilbert D. Recommendation for the assessment of tobacco craving and withdrawal in smoking cessation trials. Nicotine Tob Res 2004 Aug; 6 (4): 599-614
53. Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. Arch Gen Psychiatry 1986 Mar; 43 (3): 289-94
54. Benowitz NL. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther 2008 Apr; 83 (4): 531-41
55. Piasecki TM. Relapse to smoking. Clin Psychol Rev 2006 Mar; 26 (2): 196-215
56. Piasecki TM, Fiore MC, Baker TB. Profiles in discouragement: two studies of variability in the time course of smoking withdrawal symptoms. J Abnorm Psychol 1998 May; 107 (2): 238-51
57. Davis JA, Gould TJ. Associative learning, the hippocampus, and nicotine addiction. Curr Drug Abuse Rev 2008 Jan; 1 (1): 9-19
58. Krall EA, Garvey AJ, Garcia RI. Smoking relapse after 2 years of abstinence: findings from the VA Normative Aging Study. Nicotine Tob Res 2002 Feb; 4 (1): 95-100
59. Yang T, Fisher KJ, Li F, et al. Attitudes to smoking cessation and triggers to relapse among Chinese male smokers. BMC Public Health 2006; 6: 65
60. Kenford SL, Fiore MC, Jorenby DE, et al. Predicting smoking cessation: who will quit with and without the nicotine patch. JAMA 1994 Feb 23; 271 (8): 589-94
61. Bonese KF, Wainer BH, Fitch FW, et al. Changes in heroin self-administration by a rhesus monkey after morphine immunisation. Nature 1974 Dec 20; 252 (5485): 708-10
62. Killian A, Bonese K, Rothberg RM, et al. Effects of passive immunization against morphine on heroin self-administration. Pharmacol Biochem Behav 1978 Sep; 9 (3): 347-52
63. Orson FM, Kinsey BM, Singh RA, et al. Substance abuse vaccines. Ann N Y Acad Sci 2008 Oct; 1141: 257-69
64. Fox BS, Kantak KM, Edwards MA, et al. Efficacy of a therapeutic cocaine vaccine in rodent models. Nat Med 1996 Oct; 2 (10): 1129-32
65. Pentel PR, Malin DH, Ennifar S, et al. A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats. Pharmacol Biochem Behav 2000 Jan 1; 65 (1): 191-8
66. LeSage MG, Keyler DE, Pentel PR. Current status of immunologic approaches to treating tobacco dependence: vaccines and nicotine-specific antibodies. AAPS J 2006; 8 (1): E65-75
67. Moreno AY, Janda KD. Immunopharmacotherapy: vaccination strategies as a treatment for drug abuse and dependence. Pharmacol Biochem Behav 2009 Apr; 92 (2): 199-205
68. Matsushita H, Noguchi M, Tamaki E. Conjugate of bovine serum albumin with nicotine. Biochem Biophys Res Commun 1974 Apr 23; 57 (4): 1006-10
69. Castro A, Monji N, Ali H, et al. Nicotine antibodies: comparison of ligand specificities of antibodies produced against two nicotine conjugates. Eur J Biochem 1980 Mar; 104 (2): 331-40
70. LeSage MG, Keyler DE, Hieda Y, et al. Effects of a nicotine conjugate vaccine on the acquisition and maintenance of nicotine self-administration in rats. Psychopharmacology (Berl) 2006 Mar; 184 (3-4): 409-16
71. Pravetoni M, Keyler DE, Raleigh MD, et al. Vaccination against nicotine alters the distribution of nicotine delivered via cigarette smoke inhalation to rats. Biochem Pharmacol 2011 May 1; 81 (9): 1164-70
72. Pentel P, Malin D. A vaccine for nicotine dependence: targeting the drug rather than the brain. Respiration 2002; 69 (3): 193-7

73. Keyler DE, Roiko SA, Benlhabib E, et al. Monoclonal nicotine-specific antibodies reduce nicotine distribution to brain in rats: dose- and affinity-response relationships. Drug Metab Dispos 2005 Jul; 33 (7): 1056-61
74. de Villiers SH, Lindblom N, Kalayanov G, et al. Nicotine hapten structure, antibody selectivity and effect relationships: results from a nicotine vaccine screening procedure. Vaccine 2010 Mar 2; 28 (10): 2161-8
75. Oliver JL, Pashmi G, Barnett P, et al. Development of an anti-cotinine vaccine to potentiate nicotine-based smoking cessation strategies. Vaccine 2007 Oct 16; 25 (42): 7354-62
76. Sziraki I, Sershen H, Benuck M, et al. The effect of cotinine on nicotine- and cocaine-induced dopamine release in the nucleus accumbens. Neurochem Res 1999 Nov; 24 (11): 1471-8
77. Buccafusco JJ, Shuster LC, Terry Jr AV. Disconnection between activation and desensitization of autonomic nicotinic receptors by nicotine and cotinine. Neurosci Lett 2007 Feb 8; 413 (1): 68-71
78. Bardo MT, Green TA, Crooks PA, et al. Nornicotine is self-administered intravenously by rats. Psychopharmacology (Berl) 1999 Oct; 146 (3): 290-6
79. Matsukura S, Sakamoto N, Imura H, et al. Radioimmunoassay of nicotine. Biochem Biophys Res Commun 1975 May 19; 64 (2): 574-80
80. Roiko SA, Harris AC, LeSage MG, et al. Passive immunization with a nicotine-specific monoclonal antibody decreases brain nicotine levels but does not precipitate withdrawal in nicotine-dependent rats. Pharmacol Biochem Behav 2009 Aug; 93 (2): 105-11
81. Malin DH, Lake JR, Lin A, et al. Passive immunization against nicotine prevents nicotine alleviation of nicotine abstinence syndrome. Pharmacol Biochem Behav 2001 Jan; 68 (1): 87-92
82. Hieda Y, Keyler DE, Ennifar S, et al. Vaccination against nicotine during continued nicotine administration in rats: immunogenicity of the vaccine and effects on nicotine distribution to brain. Int J Immunopharmacol 2000 Oct; 22 (10): 809-19
83. Cerny EH, Levy R, Mauel J, et al. Preclinical development of a vaccine 'against smoking'. Onkologie 2002 Oct; 25 (5): 406-11
84. Hieda Y, Keyler DE, Vandevoort JT, et al. Active immunization alters the plasma nicotine concentration in rats. J Pharmacol Exp Ther 1997 Dec; 283 (3): 1076-81
85. de Villiers SH, Lindblom N, Kalayanov G, et al. Active immunization against nicotine suppresses nicotine-induced dopamine release in the rat nucleus accumbens shell. Respiration 2002; 69 (3): 247-53
86. Sanderson SD, Cheruku SR, Padmanilayam MP, et al. Immunization to nicotine with a peptide-based vaccine composed of a conformationally biased agonist of C5a as a molecular adjuvant. Int Immunopharmacol 2003 Jan; 3 (1): 137-46
87. de Villiers SH, Lindblom N, Kalayanov G, et al. Active immunization against nicotine alters the distribution of nicotine but not the metabolism to cotinine in the rat. Naunyn Schmiedebergs Arch Pharmacol 2004 Oct; 370 (4): 299-304
88. Keyler DE, Hieda Y, St Peter J, et al. Altered disposition of repeated nicotine doses in rats immunized against nicotine. Nicotine Tob Res 1999 Sep; 1 (3): 241-9
89. Satoskar SD, Keyler DE, LeSage MG, et al. Tissue-dependent effects of immunization with a nicotine conjugate vaccine on the distribution of nicotine in rats. Int Immunopharmacol 2003 Jul; 3 (7): 957-70
90. Hieda Y, Keyler DE, VanDeVoort JT, et al. Immunization of rats reduces nicotine distribution to brain. Psychopharmacology (Berl) 1999 Apr; 143 (2): 150-7
91. Pentel PR, Dufek MB, Roiko SA, et al. Differential effects of passive immunization with nicotine-specific antibodies on the acute and chronic distribution of nicotine to brain in rats. J Pharmacol Exp Ther 2006 May; 317 (2): 660-6
92. Carrera MR, Ashley JA, Hoffman TZ, et al. Investigations using immunization to attenuate the psychoactive effects of nicotine. Bioorg Med Chem 2004 Feb 1; 12 (3): 563-70
93. Malin DH, Alvarado CL, Woodhouse KS, et al. Passive immunization against nicotine attenuates nicotine discrimination. Life Sci 2002 Apr 26; 70 (23): 2793-8
94. Moreno AY, Azar MR, Warren NA, et al. A critical evaluation of a nicotine vaccine within a self-administration behavioral model. Mol Pharm 2010 Apr 5; 7 (2): 431-41
95. Lindblom N, de Villiers SH, Kalayanov G, et al. Active immunization against nicotine prevents reinstatement of nicotine-seeking behavior in rats. Respiration 2002; 69 (3): 254-60
96. Lindblom N, de Villiers SH, Semenova S, et al. Active immunisation against nicotine blocks the reward facilitating effects of nicotine and partially prevents nicotine withdrawal in the rat as measured by dopamine output in the nucleus accumbens, brain reward thresholds and somatic signs. Naunyn Schmiedebergs Arch Pharmacol 2005 Nov; 372 (3): 182-94
97. Malin DH, Moon WD, Goyarzu P, et al. Passive immunization against nicotine attenuates somatic nicotine withdrawal syndrome in the rat. Nicotine Tob Res 2010 Apr; 12 (4): 438-44
98. Keyler DE, Roiko SA, Earley CA, et al. Enhanced immunogenicity of a bivalent nicotine vaccine. Int Immunopharmacol 2008 Nov; 8 (11): 1589-94
99. Tuncok Y, Hieda Y, Keyler DE, et al. Inhibition of nicotine-induced seizures in rats by combining vaccination against nicotine with chronic nicotine infusion. Exp Clin Psychopharmacol 2001 May; 9 (2): 228-34
100. Roiko SA, Harris AC, Keyler DE, et al. Combined active and passive immunization enhances the efficacy of immunotherapy against nicotine in rats. J Pharmacol Exp Ther 2008 Jun; 325 (3): 985-93
101. Cornish KE, Harris AC, LeSage MG, et al. Combined active and passive immunization against nicotine: minimizing monoclonal antibody requirements using a target antibody concentration strategy. Int Immunopharmacol 2011 Nov; 11 (11): 1809-15
102. Corrigall WA. Nicotine self-administration in animals as a dependence model. Nicotine Tob Res 1999 Mar; 1 (1): 11-20
103. LeSage MG, Keyler DE, Collins G, et al. Effects of continuous nicotine infusion on nicotine self-administration in rats: relationship between continuously infused and self-administered nicotine doses and serum concentrations. Psychopharmacology (Berl) 2003 Nov; 170 (3): 278-86
104. Bevins RA, Wilkinson JL, Sanderson SD. Vaccines to combat smoking. Expert Opin Biol Ther 2008 Apr; 8 (4): 379-83
105. Trial watch: Xenova's TA-NIC vaccine shows promise. Expert Rev Vaccines. 2004 Aug; 3 (4): 386

106. Cerny EH, Cerny T. Vaccines against nicotine. Hum Vaccin 2009 Apr; 5 (4): 200-5
107. Nabi Biopharmaceuticals announces results of first NicVAX® phase III clinical trial: smoking cessation immunotherapy failed to meet primary endpoint. Rockville (MD): Nabi Pharmaceuticals, 2011
108. Gunnell D, Irvine D, Wise L, et al. Varenicline and suicidal behaviour: a cohort study based on data from the General Practice Research Database. BMJ 2009; 339: b3805
109. Ross S, Williams D. Bupropion: risks and benefits. Expert Opin Drug Saf 2005 Nov; 4 (6): 995-1003
110. Hall W. The prospects for immunotherapy in smoking cessation. Lancet 2002 Oct 5; 360 (9339): 1089-91
111. Brischoux F, Chakraborty S, Brierley DI, et al. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A 2009 Mar 24; 106 (12): 4894-9
112. Hnasko TS, Sotak BN, Palmiter RD. Morphine reward in dopamine-deficient mice. Nature 2005 Dec 8; 438 (7069): 854-7
113. Naqvi NH, Rudrauf D, Damasio H, et al. Damage to the insula disrupts addiction to cigarette smoking. Science 2007 Jan 26; 315 (5811): 531-4
114. Rose JE, Mukhin AG, Lokitz SJ, et al. Kinetics of brain nicotine accumulation in dependent and nondependent smokers assessed with PET and cigarettes containing 11C-nicotine. Proc Natl Acad Sci U S A 2010 Mar 16; 107 (11): 5190-5
115. Fagerström K. Determinants of tobacco use and renaming the FTND to the Fagerström Test for Cigarette Dependence. Nicotine Tob Res 2012; 14 (1): 75-9
116. Rose JE, Behm FM, Westman EC, et al. Dissociating nicotine and nonnicotine components of cigarette smoking. Pharmacol Biochem Behav 2000 Sep; 67 (1): 71-81
117. Hughes JR, Rose GL, Callas PW. Nicotine is more reinforcing in smokers with a past history of alcoholism than in smokers without this history. Alcohol Clin Exp Res 2000 Nov; 24 (11): 1633-8
118. Rose JE. Nicotine and nonnicotine factors in cigarette addiction. Psychopharmacology (Berl) 2006 Mar; 184 (3-4): 274-85
119. Clemens KJ, Caille S, Stinus L, et al. The addition of five minor tobacco alkaloids increases nicotine-induced hyperactivity, sensitization and intravenous self-administration in rats. Int J Neuropsychopharmacol 2009 Nov; 12 (10): 1355-66
120. Belluzzi JD, Wang R, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology 2005 Apr; 30 (4): 705-12
121. van Amsterdam J, Talhout R, Vleeming W, et al. Contribution of monoamine oxidase (MAO) inhibition to tobacco and alcohol addiction. Life Sci 2006 Oct 19; 79 (21): 1969-73
122. Barrett SP. The effects of nicotine, denicotinized tobacco, and nicotine-containing tobacco on cigarette craving, withdrawal, and self-administration in male and female smokers. Behav Pharmacol 2010 Mar; 21 (2): 144-52
123. Benowitz NL, Hall SM, Herning RI, et al. Smokers of low-yield cigarettes do not consume less nicotine. N Engl J Med 1983 Jul 21; 309 (3): 139-42
124. Caggiula AR, Donny EC, White AR, et al. Cue dependency of nicotine self-administration and smoking. Pharmacol Biochem Behav 2001 Dec; 70 (4): 515-30
125. Shiffman S, Ferguson SG, Gwaltney CJ. Immediate hedonic response to smoking lapses: relationship to smoking relapse, and effects of nicotine replacement therapy. Psychopharmacology (Berl) 2006 Mar; 184 (3-4): 608-18
126. Malaiyandi V, Sellers EM, Tyndale RF. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. Clin Pharmacol Ther 2005 Mar; 77 (3): 145-58
127. Schoedel KA, Hoffmann EB, Rao Y, et al. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. Pharmacogenetics 2004 Sep; 14 (9): 615-26
128. Benowitz NL, Perez-Stable EJ, Herrera B, et al. Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. J Natl Cancer Inst 2002 Jan 16; 94 (2): 108-15
129. Tyndale RF, Sellers EM. Genetic variation in CYP2A6-mediated nicotine metabolism alters smoking behavior. Ther Drug Monit 2002 Feb; 24 (1): 163-71
130. Schnoll RA, Patterson F, Wileyto EP, et al. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. Pharmacol Biochem Behav 2009 Mar; 92 (1): 6-11
131. Hughes JR, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. Addiction 2004 Jan; 99 (1): 29-38
132. West R, Sohal T. "Catastrophic" pathways to smoking cessation: findings from national survey. Bmj 2006 Feb 25; 332 (7539): 458-60
133. Gonzales D, Rennard SI, Nides M, et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 2006 Jul 5; 296 (1): 47-55
134. Zatonski W, Cedzynska M, Tutka P, et al. An uncontrolled trial of cytisine (Tabex) for smoking cessation. Tob Control 2006 Dec; 15 (6): 481-4
135. West R, Zatonski W, Cedzynska M, et al. Placebo-controlled trial of cytisine for smoking cessation. N Engl J Med 2011 Sep 29; 365 (13): 1193-200
136. Leader AE, Lerman C, Cappella JN. Nicotine vaccines: will smokers take a shot at quitting? Nicotine Tob Res 2010 Apr; 12 (4): 390-7
137. Lu D, Hickey AJ. Pulmonary vaccine delivery. Expert Rev Vaccines 2007 Apr; 6 (2): 213-26
138. Davis SS. Nasal vaccines. Adv Drug Deliv Rev 2001 Sep 23; 51 (1-3): 21-42
139. Hall W, Gartner C. Ethical and policy issues in using vaccines to treat and prevent cocaine and nicotine dependence. Curr Opin Psychiatry 2011 May; 24 (3): 191-6
140. Hasman A, Holm S. Nicotine conjugate vaccine: is there a right to a smoking future? J Med Ethics 2004 Aug; 30 (4): 344-5

Correspondence: *Tobias Raupach*, MD, MME, University Hospital Göttingen, Department of Cardiology and Pneumology, D-37099 Göttingen, Germany.
E-mail: raupach@med.uni-goettingen.de