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REVIEW

Biologics to treat substance use disorders: Current status and new directions

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ABSTRACT

Biologics (vaccines, monoclonal antibodies (mAb), and genetically modified enzymes) offer a promising class of therapeutics to treat substance use disorders (SUD) involving abuse of opioids and stimulants such as nicotine, cocaine, and methamphetamine. In contrast to small molecule medications targeting brain receptors, biologics for SUD are larger molecules that do not cross the blood-brain barrier (BBB), but target the drug itself, preventing its distribution to the brain and blunting its effects on the central nervous system (CNS). Active and passive immunization approaches rely on antibodies (Ab) that bind drugs of abuse in serum and block their distribution to the brain, preventing the rewarding effects of drugs and addiction-related behaviors. Alternatives to vaccines and anti-drug mAb are genetically engineered human or bacterial enzymes that metabolize drugs of abuse, lowering the concentration of free active drug. Pre-clinical and clinical data support development of effective biologics for SUD.

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The need for development of safe and effective therapies for SUD

Substance use disorders are a public health and economic threat worldwide.[1] In the U.S., there are approximately 70 million tobacco users and 21.5 million people affected from other SUD.[2,3] Abuse of tobacco products accounts for about 500,000 deaths annually causing up to 90% deaths due to lung cancer, cardiovascular, and lower tract respiratory diseases.[3] In 2014, an estimated 2.5 million people were dependent on heroin and prescription opioid analgesics, leading to a total of 29,500 deaths by overdose.[2] The yearly public cost of SUD exceeds $700 billion in criminal activities, lost work productivity, and health care expenditures.[4] Despite the societal impact of SUD, limited therapeutic options exist,[5-7] necessitating the development of new strategies for treating SUD.[8] This review broadly describes the development of biological therapies for SUD, and mainly focuses on vaccines for SUD. Detailed reviews on the various biological approaches targeting specific drugs of abuse are found elsewhere.[9-19]

Biologics for SUD: Overview

Biologics for SUD encompass a wide range of therapeutic strategies such as active immunization with vaccines that stimulate generation of polyclonal anti-drug Ab, passive immunization with anti-drug mAb, genetically modified enzymes that degrade drugs of abuse, viral-mediated transfer of genes encoding for anti-drug mAb or enzymes, and catalytic Ab that facilitate drug degradation. Approved medications for SUD are small molecules that provide pharmacodynamic-based therapy targeting CNS receptors involved in drug addiction (e.g., the opioid receptor agonist methadone, or the nicotinic receptor partial agonist varenicline). In contrast, biologics for SUD are larger molecules that do not cross the BBB, but target the drug itself. Biologics for SUD bind or degrade the free drug, preventing its distribution to the brain and blunting its central effects. Due to their pharmacokinetic-based mechanism of action, biologics for SUD may be used in combination with existing pharmacodynamic-based medications. SUD are complex and heterogeneous diseases that will benefit from combination therapies to improve overall treatment outcomes and/or from precision medicine strategies to target specific patient subsets.

Vaccines for SUD

Vaccines for SUD are immunogens consisting of synthetic drug-derived haptens chemically conjugated to immunogenic carriers and packaged in adjuvants to increase immunogenicity and provide an injectable formulation. Vaccination with these conjugate immunogens leads to T cell-dependent B cell activation to generate polyclonal anti-drug Ab that bind free drug in serum. Ab-bound drugs do not cross the BBB, thus reducing drug distribution to the brain and blocking addiction-related behaviors.[10,20] Although this approach has shown promising pre-clinical efficacy against nicotine, cocaine, methamphetamine, heroin and prescription opioids, no vaccine for SUD has yet been approved for clinical use. Vaccines for SUD could offer long-lasting, safe, and cost-effective interventions that avoid side effects associated with current addiction medications.

Clinical studies

Clinical evaluation of first-generation vaccines against nicotine and cocaine involved intramuscular injections of immunogens

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adsorbed on aluminum-based adjuvants.[21-23] Immunization schedules included 3–5 monthly injections followed by boosts every 2–6 months to maintain serum anti-drug Ab levels.[21-23] Table 1 summarizes clinical studies of vaccines, and other biologics for SUD. First-generation vaccines for nicotine and cocaine showed proof of efficacy (e.g., smoking cessation, or cocaine-free urine) only in the subset of immunized subjects (~30%) that achieved the highest anti-drug Ab concentrations (≥40 μg/ml).[21-24] Newer vaccine formulations are currently under pre-clinical and early-stage clinical development. Selecta Biosciences developed a polymer-based nanoparticle nicotine vaccine (SEL-068), which recently completed a phase I trial, but data are not yet available (NCT01478893). Pfizer has recently conducted clinical studies to test the safety, tolerability, immunogenicity, and efficacy of 2 lead nicotine immunogens, but results have not been disclosed (NIC7-001, and NIC7-003, NCT01672645). A phase I trial of a cocaine vaccine containing a disrupted adenovirus as carrier is currently recruiting patients (NCT02455479).

Clinical trials of first-generation SUD vaccines highlight the need to understand why potentially clinically effective Ab responses were achieved only in a fraction of immunized subjects, and how to improve the magnitude, quality and duration of the post-immunization serum Ab response to generate more effective vaccines.

Pre-clinical development

This section first discusses immunological mechanisms underlying generation of polyclonal anti-drug Ab responses, which may help to explain, and predict, post-immunization individual variability in vaccine efficacy against SUD. Then new components, designs, materials, and immunization strategies currently explored in pre-clinical development of next-generation vaccines for SUD are reviewed.

Immunological mechanisms underlying polyclonal Ab generation

After immunization, vaccines are processed by antigen-presenting cells (APC) displaying major histocompatibility complex II (MHC II) receptors. After presentation to B and T cell lymphocytes, generation of Ab relies on $CD4^+$ T helper (T_h) cell-

Table 1. Clinical trials and human laboratory studies of biologics for SUD.*

Biologic	SUD	Description	Company/ Sponsor	Phase	NCT/ Reference	Development Status
Vaccine	Nicotine	3′-Aminomethyl-nicotine-rEPA (NicVax)	Nabi Pharmaceuticals (acquired by GSK)	I-III	NCT01102114, NCT01304810 NCT00598325, NCTNCT00218413 NCT00836199, NCT00318383 [22,176]	Halted
		NicVax plus varenicline	Maastricht University Medical Center	IIb	NCT00995033, [177,178]	—
		NicVax	Maastricht University Medical Center	I-II (fMRI)	NCT01318668	—
		NicVax	Yale University/Nabi/NIDA	Lab (SPECT)	NCT00996034, [179]	—
		Nicotine hapten-VLP from Bacteriophage Qβ (NicQβ, CYT002, NIC002)	Cytos (acquired by Novartis)	I-IIb	NCT01280968, NCT00736047 NCT00369616	Halted
		Nicotine hapten-TT (Niccine)	Independent Pharmaceutical	II	[180]	Halted
		Nicotine hapten-rCTB (TA-NIC)	Celtic Pharma (Xenova)	I-II	NCT00633321	Halted
		NIC7–CRM_{197} alum/CpG (NIC7-001 and −003)	Pfizer	I	NCT01672645	—
		Polymer-based nanoparticle containing TLR ligands (SEL-068)	Selecta Biosciences	I	NCT01478893	Ongoing (reformulation)
	Cocaine	Succinylnorcocaine-rCTB (TA-CD)	Celtic Pharma (Xenova)/ Baylor College of Medicine	I, IIa-b, III	NCT00969878, [23,181]	—
		TA-CD	New York State Psychiatric Institute	Lab, II	NCT00965263, [182]	—
		Cocaine hapten-dAd5 (dAd5GNE)	Weill Medical College of Cornell University	I	NCT02455479	Ongoing
mAb	Meth	Chimeric mAb7F9	InterveXion Therapeutics	I	NCT01603147, [146]	Ongoing
Enzyme	Cocaine	BChE: recombinant human serum albumin (HSA) mutated butyrylcholinesterase (AlbuBChE, TV-1380)	Teva Pharmaceutical Industries	I-II	NCT01887366, [148,150]	Ongoing
		Double mutant CocE (RBP-8000)	Indivior Inc.	I-II	NCT01846481, [149]	Ongoing

*Available publication or clinical trial registration (www.clinicaltrials.gov). Virus-like particle (VLP), recombinant exotoxin A Pseudomonas *aeruginosa* (rEPA), recombinant Cholera Toxin B (rCTB), tetanus toxoid (TT), disrupted adenovirus type 5 (dAd5), human butyrylcholinesterase (BChE), bacterial cocaine esterase (CocE), single-photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI). Symbol: "—" not applicable or no information available at this time.

dependent B cell activation in germinal centers (GC), within the lymph nodes and spleen.[25-30] In the GC, antigen-specific B cells go through isotype switching, affinity maturation and clonal selection.[29,30] B cell maturation and differentiation in the GC are supported by T follicular helper (T_{fh}) cells and GC-T_{fh}, which are T_h subsets uniquely specialized for B cell help.[31,32] Germinal center formation is essential for generating long-lived high-affinity plasma cells and switched immunoglobulin memory B cells.[25-30] This series of cellular and molecular events is critical for generating long-lasting high-affinity antigen-specific Ab.

a) B and T cell lymphocyte responses to SUD vaccines. Vaccines for SUD consist of drug-derived haptens (B cell epitope) conjugated to larger foreign immunogenic carriers (e.g., proteins or peptides) that provide signaling for activation of T cells (T cell epitope). Characterization of hapten-specific B cells and carrier-specific T cells can help elucidate the cellular and molecular mechanisms underlying generation of effective anti-drug Ab responses in immunized subjects.

To address this question, fluorescent antigen-based magnetic enrichment paired with flow cytometry allows analyses of polyclonal antigen-specific B and T cell populations.[33,34] The strength of this approach is that very rare antigen-specific B and T cells are detected prior to, or shortly after immunization.[35-39] To date, this approach has been used to test the effect of hapten structure,[40,41] adjuvant,[42] or host genetics[42] on the B cell response to vaccines for SUD. This strategy has also been used to study the relationship between antigen-specific B and T cells and individual variability in post-immunization Ab titers, or efficacy against drug distribution and drug-induced behavior in mice.[41,43] These studies found that naïve and early-activated B cells can discriminate between structurally-related haptens, and that the size of the polyclonal hapten-specific B cell population determines vaccine immunogenicity and efficacy against drugs of abuse.[40,41] When comparing individuals, differences in the population size of hapten-specific B cells and carrier-specific T cells are found before and/or after immunization in individual mice, and correlate to vaccine immunogenicity and efficacy.[41,43] Hence, analysis of naïve and early-activated B and T cells could be used to examine vaccine formulations and individual variability across subjects.

Development of SUD vaccines has made use of serum Ab subclasses analysis to test whether specific vaccine formulations induced post-immunization anti-drug Ab more characteristic of a T_h1- (IgG_{2a} and IgG_3 subtypes) or a T_h2- (IgG_1) response.[44-47] Yet, it is not fully understood whether immunization against drugs of abuse benefits from T_h2-polarized or more balanced T_h1/T_h2 responses.[44-47] Immunogen dose and adjuvant choice are known to affect $CD4^+$ T cell clonal expansion, differentiation, and polarization.[48-50] It is possible that specific polarization patterns in the $CD4^+$ T cell repertoire (T_h1 v. T_h2, or T_h1 v. T_{fh}[48,39,51]) are associated with increased efficacy of vaccines for SUD. Vaccine formulations could be screened for their ability to induce desired antigen-specific $CD4^+$ T cell subsets (e.g., T_{fh} and GC-T_{fh}[31,32]) known to help GC B cell activation, and generation of high-affinity Ab.

b) Frequency of naïve and early-activated vaccine-specific B and T cell subsets correlates to individual vaccine efficacy. Pre-clinical and clinical studies of SUD vaccines showed that post-immunization anti-drug Ab levels and affinity vary greatly across subjects, but the cause of such variability is not clear.[10] Before and after immunization, the number of antigen-specific B and T cells in the total lymphocyte repertoire varies in individual mice[33,36-39,52-58] and in human subjects.[59,60] Consistently, individual variability of similar magnitude has been found in the polyclonal hapten-specific B cell population in blood, peripheral lymph nodes and spleen of various mouse strains, before and after immunization with vaccines for oxycodone and nicotine.[40-43] Multiple mechanisms may underlie the pre- and post-immunization variability in the antigen-specific B and T cell subsets and contribute to individual vaccine efficacy. Apoptosis and antigen affinity affect the heterogeneity of the primary immune response by limiting differentiation of a single naïve B cell shortly after immunization.[38] Clonal selection and affinity for antigen affect the antigen-specific naïve T cell population size, heterogeneity, and its differentiation into specialized subsets after immunization or infection.[36,39,48,51,54-56] These data suggest that optimal activation of both antigen-specific naïve B and T cell populations is a key process for achieving clinically effective Ab responses, and support the hypothesis that variations in the population size of vaccine-specific B or T cells underlie individual efficacy of SUD vaccines.

B cells. The frequency of naïve and/or early-activated hapten-specific B cells correlated post-immunization Ab responses and vaccine efficacy against oxycodone[40,43] and nicotine.[41] The size of the polyclonal hapten-specific IgM^{high} naïve and memory B cell and GC B cell subsets best correlated with post-vaccination serum anti-drug IgG Ab titers and vaccine efficacy against drug distribution or drug-induced behavior in mice.[41,43] A greater number of pre-immunization hapten-specific IgM^{high} naïve B cells correlated to increased post-immunization serum anti-oxycodone Ab titers, and efficacy in blocking oxycodone distribution to the brain.[43] These data suggest that variations in the pre-immunization polyclonal hapten-specific B cell repertoire underlie individual responses to vaccines for SUD. Immunization with an oxycodone vaccine increased GC activation over time, and the extent of GC activation was highly correlated with individual vaccine efficacy in mice.[43] These data suggest that variations in the magnitude and quality of GC formation underlie individual vaccine efficacy. New immunization strategies that enhance GC activation shall be adopted to increase efficacy of vaccines and the fraction of subjects achieving clinically effective anti-drug Ab.

T cells. The carrier is a key component of conjugate immunogens for its role in stimulating specific subsets of cognate $CD4^+$ T cells to help hapten-specific B cell activation.[32] Selective depletion of $CD4^+$ T cells blunted clonal expansion of hapten-specific B cells and vaccine efficacy against oxycodone, suggesting that the magnitude of $CD4^+$ T cell activation is key for adequate post-vaccination B cell and Ab responses.[43] Using a magnetic enrichment strategy involving use of T cell epitope peptides covalently bound to soluble MHC II receptors labeled with fluorescent proteins, it was shown that greater frequency of carrier-specific MHC II-restricted $CD4^+$ T cells prior to immunization correlated to increased post-immunization efficacy in retaining serum oxycodone.[43] These data highlight the importance of carrier-specific $CD4^+$ T cells in contributing to the vaccine efficacy against SUD. Consistently, nanoparticle

nicotine vaccines containing MHC II peptide sequences that stimulate $CD4^+$ T cells elicited effective anti-nicotine Ab in mice and non-human primates (NHP), and stimulated recall T cell memory in human peripheral blood mononuclear cells (PBMC) *ex vivo*.[61]

c) Vaccine-specific B and T cells are biomarkers predictive of vaccine efficacy. A greater frequency of vaccine-specific B or T cells correlates with more effective immunization against SUD.[40,41,43] Early appearance of anti-oxycodone Ab predicted vaccine efficacy in blocking oxycodone distribution to the brain in mice (Fig. 1A and B, reproduced from ref. 43). However, the frequency of hapten-specific B cells in blood prior to, and after immunization was also predictive of vaccine efficacy (Fig. 1C and D, reproduced from ref. 43). Consistently, the frequency of early hapten-specific B cell responses predicted the immunogenicity and efficacy of nicotine vaccines containing structurally different haptens.[41] These data suggest that analysis of vaccine-specific B or T cells prior to, or shortly after immunization could be used to anticipate clinically significant individual responses to vaccination against SUD, which will greatly facilitate adaptive trial design, patient stratification, and personalized medicine. Analysis of hapten-specific B cells prior to immunization could be paired to analyses of genetic markers predictive of individual responses to vaccination.[62-64] For instance, early screening of vaccine-specific B and T cells in PBMC could be combined with other behavioral, biochemical or genetic tests to predict whether individual, or subsets of, SUD patients will be more likely to benefit from vaccination, pharmacotherapy, or a combination of vaccination and medication.

Vaccine design

a) Improving individual vaccine components: hapten, carrier, and adjuvant. Pre-clinical studies have examined the role of hapten and linker design, bioconjugation chemistry, carrier, and adjuvant to improve the anti-drug Ab response and vaccine efficacy.[10,20,65-68]

Hapten, linker, and bioconjugation chemistry. Drug-based haptens are key components for generating drug-specific Ab. Hapten development is a complex process of screening hapten series containing various chemical modifications and linkers placed at different positions on the target drug molecule, and optimization of lead hapten synthesis and bioconjugation chemistry for coupling to carriers. For instance, increased

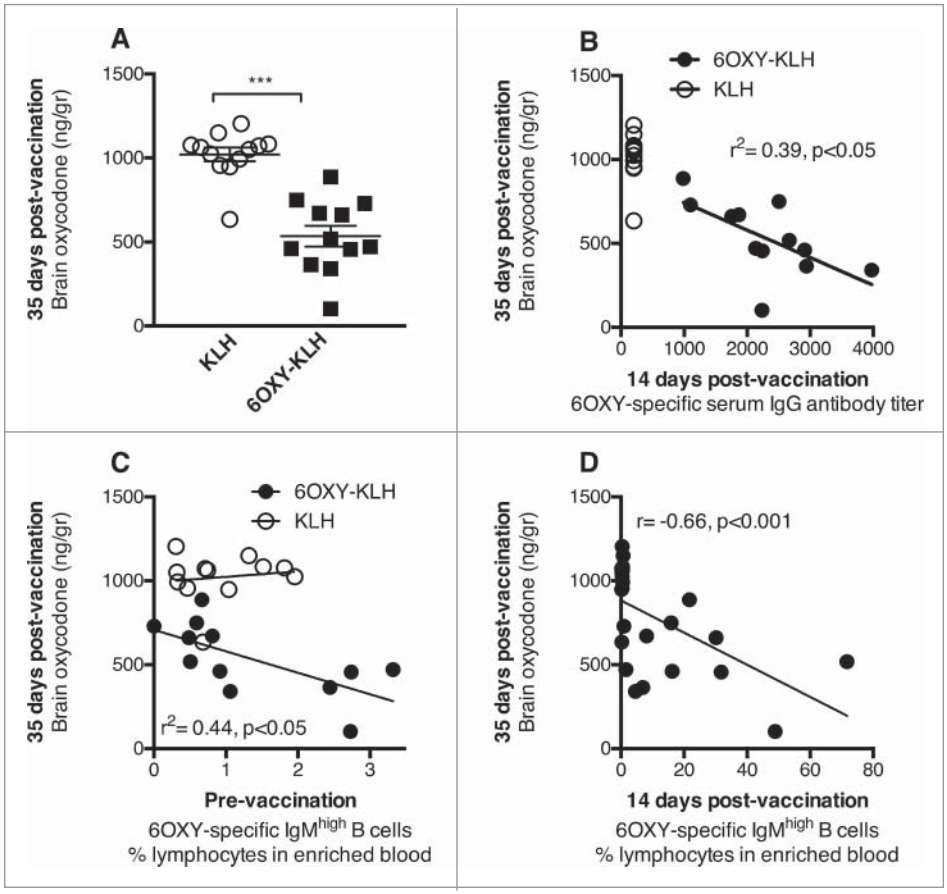

Figure 1. The frequency of hapten-specific B cells in blood predicts vaccine efficacy. Pre- and post-vaccination 6OXY-specific B cells were analyzed in 0.2 ml of blood collected before and after immunization with either 6OXY-KLH or KLH. Balb/c mice were immunized on days 0, 14 and 28, and challenged with 2.25 mg/kg oxycodone a week after the third immunization to measure the effect of immunization on oxycodone distribution. A) Immunization reduced distribution of oxycodone to the brain. Data are mean ± SEM. B) Early serum IgG antibody titers correlated to subsequent blockage of oxycodone distribution to the brain. C) The frequency of 6OXY-specific IgM^{high} B cells prior to immunization correlated with vaccine efficacy in the 6OXY-KLH group. D) Increased frequency of 6OXY-specific IgM^{high} B cells 14 days after the first immunization correlated to greater vaccine efficacy on oxycodone distribution to the brain. Frequencies are the % of total lymphocytes in the bound fraction after positive enrichment of blood. Data include 3 independent experiments with a total of n = 12 mice each group. *** $p < 0.001$ compared to KLH control. *Copyright 2015. The American Association of Immunologists, Inc.*

hapten stability correlated to higher post-immunization anti-cocaine Ab and vaccine efficacy.[69] Hapten enantiopurity has been shown to improve quantity and quality of post-vaccination Ab specific for (−)nicotine, suggesting that enantiopure haptens should be favored over racemic mixtures to generate immunogens for clinical evaluation.[70] Additionally, hapten fluorination,[71] hapten clustering,[72] and conformationally-constrained hapten structures[73] have been explored as potential strategies to design more effective haptens. These data suggest that continuous efforts on hapten synthesis will generate more chemically defined and effective haptens.

A candidate vaccine for prescription opioids oxycodone and hydrocodone consists of an oxycodone-based hapten containing a tetraglycine linker at the C6 position (6OXY$(Gly)_4$OH) and conjugated to keyhole limpet hemocyanin (KLH) by carbodiimide chemistry.[74-76] The 6OXY$(Gly)_4$-KLH conjugate was more effective in blocking oxycodone distribution to the brain and oxycodone-induced antinociception than vaccines consisting of C6-oxycodone haptens conjugated to KLH through an hemisuccinate linker[74] or thiol-maleimide bondage.[75] The 6OXY$(Gly)_4$-KLH was also more effective in blocking hydrocodone distribution to the brain and hydrocodone antinociception than immunogens containing C6- and C8-derivatized hydrocodone haptens.[75] Other positions on oxycodone and hydrocodone still remain to be explored. In contrast, development of heroin vaccines explored a wider range of hapten structures, generated from derivatization of the morphinan structure at the C3 and C6 position,[77-82] and at the bridgehead nitrogen[83,84] (reviewed in detail elsewhere[11,19]). Although these series of opioid-based immunogens provided a comprehensive and informative structure-function dataset, data interpretation needs to account for differences in conjugation chemistry, haptenization ratio, and hapten or carrier structure.

Optimization of lead immunogens benefits from screening structurally-similar haptens paired with different bioconjugation chemistry (e.g., carbodiimide v. maleimide coupling) to improve hapten density, and vaccine immunogenicity or efficacy.[75,85-87] Increased efficacy of a heroin vaccine correlated with higher haptenization ratios in conjugates containing a tetanus toxoid (TT) carrier but not the diphtheria toxin cross-reactive material (CRM_{197}) carrier.[88] Yet, haptenization ratio did not correlate with anti-heroin Ab titer nor affinity.[88] These data suggest that each hapten-conjugate combination needs to be tailored to specific formulations or immunization regimens, and evaluated for multiple parameters of immunogenicity and efficacy.

A large series of nicotine-based haptens containing linkers of different length, polarity, and flexibility placed at various positions on the nicotine structure was tested for immunogenicity and function.[87] This impressive effort yielded a lead immunogen consisting of the 5-aminoethoxy-nicotine (Hapten 7) conjugated to CRM_{197}.[87] The optimized NIC7–CRM_{197} adsorbed on alum plus the toll-like receptor (TLR) 9 agonist CpG adjuvant showed promising pre-clinical efficacy in both mice and NHP,[46,87,89] and these candidates were recently evaluated in a clinical trial (Table 1). Analysis of several lots of NIC7–CRM_{197} showed that higher vaccine immunogenicity and efficacy correlated to lower degree of conjugate aggregation, and minimal presence of adducts.[89] Also, NIC7–CRM_{197} conjugates with haptenization ratios between 11 and 18 showed best nicotine-specific Ab levels, affinity for nicotine, and function.[89]

In summary, hapten chemistry, linker composition and position, bioconjugation chemistry, hapten load, and conjugate purity are important parameters that affect immunogenicity and efficacy of vaccines for SUD.

Carrier. Vaccines for SUD have employed TT, KLH, Cholera Toxin B, recombinant exotoxin A from *Pseudomonas aeruginosa* (rEPA), CRM_{197}, virus-like particles (VLP), or peptide-based carriers.[90-92]

To identify the most promising carrier suitable for translation of a vaccine against oxycodone and hydrocodone, BSA, decamer and dimer KLH, TT, and a TT-derived peptide were tested.[40,42,43,74-76,93] To develop vaccines for treatment of methamphetamine abuse, for which no therapies exist, immunogens containing either native or dimer KLH, or TT, showed promising pre-clinical efficacy in blocking distribution of methamphetamine to the brain and blocking a variety of methamphetamine-induced behaviors, including intravenous self-administration.[94-98]

Other carrier proteins have adjuvant-like properties, such as the well-characterized CRM_{197}.[99] A cocaine hapten conjugated to the TLR5 agonist flagellin elicited greater cocaine-specific Ab concentrations than the same hapten conjugated to KLH.[100] A novel nicotine vaccine employed a peptide-based trimeric coiled-coil (TCC) carrier containing $CD4^+$ T cell epitopes.[101] The TCC-based vaccine was delivered with alum plus an additional adjuvant formulation containing the TLR4 agonist glucopyranosyl lipid and a stable oil-in-water emulsion.[101] The TCC carrier displayed a higher density of lysines compared to KLH, rEPA, TT or CRM_{197}, permitting higher hapten load. The TCC-based vaccine containing 12 nicotine haptens per trimer performed better than a KLH-conjugate containing 22 nicotine haptens per monomer, but performed similarly to a KLH-conjugate displaying higher hapten density.[101]

Conjugate nicotine and cocaine vaccines have made use of disrupted adenoviruses as carriers, and showed pre-clinical efficacy in various animal models including NHP.[102-105] In this approach, the recombinant $E1^-$ $E2^-$ replication-deficient serotype 5 adenovirus (Ad5) is disrupted by a combination of chemicals and heat, and its capsid proteins conjugate to haptens.[102,103] A cocaine vaccine containing the Ad5-based carrier is now advanced toward clinical evaluation (Table 1).

In sum, use of better characterized and more immunogenic carrier proteins with adjuvant-like properties will provide more effective vaccines for SUD. Development of more structurally defined carriers (see next section for particle-based scaffolds) will also improve control of hapten-to-carrier ratio and provide immunogens suitable for pharmaceutical manufacturing and scale-up.

Adjuvant. The most commonly used adjuvants for pre-clinical development of vaccines for SUD have been Freund's complete and incomplete adjuvant, aluminum, the TLR4 agonist monophosphoryl lipid A (MPLA), and the TLR9 agonists class B CpG oligodeoxynucleotides, containing either a phosphodiester or a modified nuclease-resistant phosphorothioate backbone.[67] Additional adjuvant options for SUD vaccines include: AS01, a combination of liposomes containing MPLA and QS21

saponin; AS03, an oil-in-water emulsion containing α-tocopherol, squalene, and polysorbate 80; and AS04, a combination of aluminum hydroxide and MPLA.[67] Development of SUD vaccines could also benefit from testing other adjuvants of interest, which are at different stages of development or regulatory approval.[106] For instance, the Food and Drug Administration (FDA) recently approved MF59, a squalene-based oil-in-water emulsion used in influenza vaccines, which promotes GC B cell and T_{fh} cell responses,[52,107] key features for generation of high-affinity Ab.

Adjuvants, or adjuvant combinations, have been tailored to specific immunogens to determine the best formulation. For instance, an oxycodone vaccine was effective in Freund's and alum adjuvants,[40,42,43,74-76,93] but not MPLA.[42] The combination of alum and CpG with a candidate vaccine against nicotine improved Ab titer, affinity, nicotine binding capacity, and Ab efficacy in blocking nicotine distribution to the brain, compared to immunization with the same vaccines adsorbed on alum only, in mice and NHP.[46,108] Similarly, addition of phosphorothioated CpG to a heroin vaccine adsorbed on alum enhanced Ab titer, affinity, blockage of opioid-induced antinociception.[45] Combination of CpG and alum also showed preclinical efficacy for vaccines directed against the prescription opioid fentanyl.[109] These data suggest that addition of CpG to alum adjuvant improves the quantity, quality, and function of the post-vaccination Ab response, and that this approach will likely improve other vaccines for SUD.

Combinations of vaccines and adjuvants should also be evaluated for immunogenicity and efficacy by different routes of administration.[42,45] Intradermal immunization with a nicotine vaccine combined with skin illumination by a laser source, which increases the motility of dendritic cells,[110] showed immunogenicity and efficacy comparable to intramuscular immunization with the same vaccine adsorbed on alum, MPLA, and MPLA/CpG.[111] Although translation of new adjuvants is often limited by toxicity, side effects, proprietary restrictions, or regulatory approval, it will be important to test available pre-clinical stage, licensed, and/or non-traditional adjuvants to constantly improve the efficacy of SUD vaccines.

b) Particle-based vaccines for SUD: new materials. Particles provide flexible platforms for delivery of vaccines and adjuvants, and offer the unique advantage of building defined vaccine scaffolds. Micro- and nano-particles are built from polymers, liposomes, VLP, self-assembly peptides, and complex combinations of other materials.[112-114] In contrast to soluble formulations, particle-based delivery is expected to enhance vaccine immunogenicity and efficacy by providing enhanced APC stimulation, precise stoichiometry or geometry of vaccine components, controlled hapten load, focused delivery at injection site of adjuvants or other immunomodulators, and creation of a depot effect for sustained release.

VLP. Virus-like particles are known to promote memory B cell differentiation and induced $CD4^+$ T cell help.[115,116] A nicotine vaccine using a VLP scaffold derived from the bacteriophage Qβ advanced to clinical evaluation (Nic-Qβ).[21] Similarly to other first-generation nicotine vaccines, Nic-Qβ showed efficacy only in the fraction of subsets that achieved the highest anti-nicotine Ab levels.[21] A malaria vaccine containing *Plasmodium falciparum* epitopes displayed on the surface of VLP from hepatitis B and administered in AS01 adjuvant showed clinical efficacy in phase III trials,[117,118] received regulatory approval from the European Medicines Agency (EMA), and is now the world's first licensed malaria vaccine (Mosquirix®). These data indicate that VLP are safe and effective scaffolds capable of inducing long-lasting immune responses, suggesting that more VLP-based formulations should be evaluated in SUD vaccines.

Liposomes. Use of liposomes, or their combination with other materials, is a general and versatile approach that could generate novel scaffolds for SUD vaccines. Strategies for development of SUD vaccines have included: heroin immunogens combined with, or conjugated to, liposomes containing MPLA[90]; a methamphetamine vaccine combined with liposomes containing a lipid analog of the immunomodulator tucaserol[119]; and nicotine immunogens conjugated to an adjuvanted lipid vesicle platform.[120] A novel nicotine vaccine was built from nicotine-BSA conjugated to a liposome-based particle, and described as a lipoplex complex.[121] A second-generation lipoplex contained a carbon-based nanoparticle backbone, called a nanohorn, that increased the stability of the liposome structure,[47] and showed greater nicotine-specific Ab titers than the nicotine-BSA conjugated to lipoplexes without the nanohorn scaffold.[47] It will be of interest to test whether other adjuvants, immunomodulators, or carriers could be incorporated into liposomes or liposome-based nanoparticles.

Polymers. Selecta Bioscience's nanoparticle nicotine vaccine (SEL-068) consists of polymers conjugated to nicotine haptens and TLR ligands, and contains immunogenic peptides (Table 1). The SEL-068 has shown pre-clinical efficacy in mice and NHP,[61,122] selectively blocking nicotine discrimination and decreasing behavioral effects of nicotine in squirrel monkeys.[92] The effect of immunization was greater in naive than nicotine-experienced squirrel monkeys, suggesting that immunization approaches may be more effective in treating light smokers, subjects at early stages of tobacco addiction, or those at risk of relapse.[123] The Selecta Bioscience group also showed that polymer encapsulation increased adjuvant properties of a TLR ligand and immunogenicity of a model antigen, and reduced the likelihood of adjuvant systemic effects.[124] These data support further studies to explore whether polymer-based vaccine designs or delivery platforms will generate more effective vaccines for SUD.

Other strategies. Approaches for improving antigen presentation include DNA-protein structures,[125] self-assembled peptide-based nanofibers,[126,127] and coat glycoproteins of the T. *brucei* parasite.[128] These approaches incorporate cutting-edge materials and delivery systems that are being investigated in vaccines for other indications (e.g., carbohydrate vaccines[129]), and that can be harnessed to design micro- and nano-particle vaccines for SUD. Vaccine physical-chemical characteristics, including size, molecular patterns, and geometry play a role in vaccine delivery and efficacy.[130] Hence, biomaterials can be used to rationally design vaccines that exploit pathogen's features including spatially organized antigens to stimulate B cell receptor (BCR) cross-linking, and display of appropriate signals to stimulate APC uptake, and antigen processing.[131] Alternatively, synthetic vaccines can be functionalized for targeted delivery to lymph nodes and GC activation.[132,133] Although particles and new materials allow for development of more

structurally defined vaccines, it is of interest to test whether their efficacy will be superior to more traditional hapten-protein conjugate immunogens.

Immunization strategies

To increase a vaccine's efficacy, it is possible to co-administer different vaccines in multivalent immunization strategies. A pediatric trivalent vaccine targets tetanus, diphtheria, and acellular pertussis (TDaP®), while a 23-valent pneumococcal polysaccharide vaccine (Pneumovax®) is effective against 23 serotypes of *S. pneumonia*. Multivalent immunization strategies have also shown pre-clinical efficacy against nicotine and opioids.[93,134] Nicotine vaccines containing structurally-related haptens elicit non-overlapping anti-nicotine Ab responses, possibly through activation of distinct hapten-specific B cells populations.[86] To target heroin and prescription opioids, co-administration of an oxycodone vaccine with an analogous morphine vaccine blocked both oxycodone and the active heroin metabolite 6-monoacetyl-morphine (6-MAM) in rats.[93] Additionally, an unexpected synergistic effect of bivalent immunization elicited greater anti-opioid Ab titers, and efficacy to block oxycodone and 6-MAM distribution compared to individual immunogens.[93] These data suggest that combining different vaccines may achieve greater efficacy against the target drug(s), and that multivalent immunization strategies increase vaccine efficacy, maintain selective blockade of specific compounds, and provide protection against multiple compounds. This approach may generalize to other SUD.

General considerations for development and translation of vaccines for SUD

Selection criteria for next-generation vaccines

Although clinical evaluation of first-generation vaccines set benchmarks for the minimum requirements of clinically effective anti-drug Ab responses, there are no absolute criteria for successful immunization against SUD. Measurement of anti-drug Ab titers is a well-established parameter of immunogenicity, but methodological differences between laboratories or vaccine formulations complicate comparisons. Pre-clinical development plans should include stringent and standard go/no-go criteria of vaccine immunogenicity and efficacy. Screening of new vaccine candidates should include analyses of anti-drug serum Ab titer or concentration, binding/ avidity/ affinity/ selectivity for the target drug, or other measures of Ab function. Characterization of the anti-drug Ab subclasses and analysis of post-immunization B and T cell population subsets will provide additional insights into the mechanisms underlying the efficacy of specific vaccine formulations. Vaccine candidates displaying promising immunogenicity profiles should be tested for efficacy in adequate pre-clinical models of SUD. Screenings should include testing the effect of immunization on drug distribution to the serum and to the brain, drug-induced behaviors, or drug-induced alteration of brain neurochemistry. To increase the likelihood of translation, lead vaccines should also display pre-clinical efficacy across strains or species. A detailed review of animal models for screening of candidate vaccines for SUD can be found elsewhere.[135]

Realistic expectations for vaccines for SUD

Although vaccines represent one of the most effective interventions introduced in medical practice,[136] no vaccine is 100% effective. Based on the track record of vaccines for infectious diseases, it is not realistic to expect that vaccines for SUD, or other non-communicable chronic diseases, will be effective in all immunized subjects. To increase the likelihood of successful translation of SUD vaccines, a few considerations need to be made: (1) Most vaccines for infectious diseases are prophylactic interventions administered in neonates, infants and small children to prevent infection or block toxin activity when exposed. Non-standard immunization regimens are also administered to at-risk subjects prior to exposure. Vaccines for tropical diseases (e.g., yellow fever, or Japanese encephalitis) and biological warfare agents (e.g., Biothrax against *Bacillus antracis* toxin) are administered prior to travel, or deployment of military personnel in endemic areas. In contrast, therapeutic vaccines for SUD are tested in current drug users, when the concentration of drug in serum and brain greatly exceeds the number of drug-specific binding sites on Ab (i.e., stoichiometric ratio of drug>biologic). It would be more practical to test their clinical efficacy in preventing early escalation of drug use after an initial exposure, or relapse after a period of abstinence. Clinical use of SUD vaccines could also benefit selected at-risk populations, younger addicts in the early stages of drug abuse, or people who have quit, and are at-risk of relapse. (2) Most vaccines for infectious diseases rely on herd immunity, which significantly reduces the overall possibility of transmission. In contrast, vaccines for non-communicable diseases do not benefit from herd immunity. Therefore, their efficacy depends exclusively on individual responses. (3) In clinical trials of vaccines for SUD, analyses of B and T cells in blood could be performed before and after immunization to guide patient stratification and predict individual vaccine responses. Biomarkers predictive of vaccine efficacy could improve therapeutic outcomes by identifying patients most likely to benefit from vaccination. (4) Clinical-stage vaccines for SUD have been tested in adults using 3–5 monthly injections to quickly stimulate high levels of anti-drug Ab. In contrast, most vaccines for infectious diseases are administered to children and adults using longer immunization schedules (see http://www.cdc.gov/vaccines) to stimulate development of long-term high-affinity memory and plasma B cells over time. Vaccines for SUD should be tested through various immunization regimens, including schedules similar to vaccines for infectious diseases to increase the likelihood of generating high-affinity long-lived plasma and memory B cells.

Other biologics for SUD

Clinical studies

mAb

The challenges of achieving post-vaccination clinically effective serum polyclonal Ab levels sufficient to effectively curb SUD may be overcome by delivering high doses of high-affinity anti-drug mAb. Administration of anti-drug mAb is a strategy for treating drug dependence or overdose that has shown pre-clinical proof of efficacy against nicotine,[137,138] cocaine,[139-141] phencyclidine,[142] methamphetamine,[143] and heroin.[144,145] The first

human study of the anti-methamphetamine chimeric mAb7F9, 0.2–20 mg/kg i.v., showed safety (NCT01603147, Table 1), and reported mAb's half-life of 17–19 days.[146] The authors also detected anti-mAb auto-Ab, a sign of immunogenicity, in 4 out of 32 subjects, but concluded that more studies were needed to understand their biological relevance or their impact on the function of anti-methamphetamine mAb.[146] To facilitate FDA approval, it will be useful to characterize recombinant humanized anti-drug mAb for presence of post-translational modifications, resulting in protein heterogeneity, that may potentially affect *in vivo* efficacy.[147] Although clinical data show promise for mAb-based therapy for SUD, long-term treatment of SUD will require multiple mAb doses throughout the entire course of therapy and may still be limited by cost. Monoclonal Ab are currently the most expensive class of biologics, but their costs will likely decrease as more effective manufacturing technologies are developed and more mAb are granted regulatory approval.

Enzymes

Another class of biologics for SUD consists of delivering enzymes that quickly metabolize or degrade the target active drug, thus preventing its neuropharmacological and behavioral effects.[9,18] Cocaine-degrading enzymes derived from human plasma butyrylcholinesterases (BChE) and bacterial cocaine esterases (CocE) have been tested for treatment of cocaine dependence and overdose (NCT01887366, and NCT01846681, Table 1).[148-150] These clinical studies showed no evidence of side effects, and provide proof of concept for enzyme-based SUD treatments. A detailed review of clinical studies of cocaine esterases can be found elsewhere.[17] Similar to mAb, enzyme-based therapies are not as limited by medication dose as vaccines. Enzymes may be more cost-effective than mAb, but no projected costs per dose have been currently disclosed.

Pre-clinical development

Passive immunization

Although the first-generation of anti-drug mAb made use of classic hybridoma technology, newer strategies can generate cost effective and longer lasting mAb, and more efficient delivery systems. To improve mAb half-life, a nanotechnology-based approach generated anti-methamphetamine mAb conjugated to dendrimers, known as dendribodies, which showed proof of pre-clinical efficacy.[151] Antigen-specific mAb can also be directly isolated from B cells of vaccinated subjects. Using various strategies, antigen-specific B cells are isolated from PBMC by cell sorting and their BCR is sequenced, cloned, and expressed in mammalian cell systems to produce antigen-specific mAb.[152-154] This approach has been used to produce mAb against infectious diseases,[153] but also to isolate high-affinity anti-nicotine mAb.[155] Isolation of anti-drug mAb from human B cells is a general approach, and therefore applies to other SUD.

Another approach to improve efficacy of passive immunization is to combine anti-drug mAb with medications or other biologics. An anti-nicotine mAb combined with the nicotine receptor antagonist mecamylamine showed a synergistic effect for blocking nicotine drug discrimination, a behavioral task that measures the subjective effects of drugs of abuse.[156] Combination of anti-drug mAb and antagonists could lower the costs associated with mAb, or reduce medications' side effects by reducing the dose of mAb or drug required to achieve clinical efficacy. Anti-drug mAb can also be combined with vaccines to enhance overall efficacy of both approaches.[157,158] These data suggest that biologics are well-suited for combination therapy.

Enzymes

Clinical studies of cocaine-degrading BChE and CocE (Table 1) provided proof of concept for using enzymes as therapeutics for treating SUD. To speed translation and improve clinical efficacy, it will be important to develop methods for production of longer lasting, cost effective, and high activity cocaine-degrading enzymes. Plants may provide a solution to scale-up production of cocaine-hydrolyzing variants of BChE.[159] Rational design of amino acid mutations and *in silico* approaches also guided design of BChE mutants with longer half-life or greater catalytic efficiency against cocaine.[160,161] Similarly, polymer-based modifications increased the half-life of a bacterial CocE.[162] These studies offer a snapshot of the numerous strategies employed to improve the efficacy of BChE and CocE.

Co-administration of cocaine hydrolases with cocaine vaccines showed additive, and possibly synergistic, effects in pre-clinical models.[163] These data support the validity of combining biological approaches acting through different mechanisms, and to further explore this approach against other SUD.

A nicotine-degrading bacterial enzyme, the flavin-dependent NicA2, has also been identified and isolated from strains of *Pseudomonas putida* found in tobacco-cultivated soil.[164] Biochemical characterization of NicA2 revealed promising stability at physiological conditions, and showed nicotine-degradation *ex vivo*.[164] Further in-depth studies involving enzyme engineering to improve catalytic activity against nicotine, and *in vivo* testing in pre-clinical models of nicotine addiction, are warranted. In sum, pre-clinical and clinical studies showed that use of genetically engineered enzymes is a promising approach for treatment of SUD, which could also be combined with other biological or pharmacological approaches to increase overall treatment outcome.

Catalytic Ab, and other biologics

Antibodies directed against transition-state analogs of cocaine catalyze the non-enzymatic hydrolysis of the cocaine benzoyl ester group, and are dubbed catalytic Ab.[165] From this first report,[165] a variety of strategies to induce catalytic polyclonal Ab through active immunization, or deliver catalytic mAb was examined.[18] Use of catalytic Ab has also been explored for methamphetamine[166] and nicotine.[167] In contrast to enzymes such as BChE or CocE, it has been challenging to further improve activity of catalytic Ab because their mechanism of action is to stabilize the transition state in non-enzymatic cocaine hydrolysis.[9,18] As a cautionary warning for readers, this section highlights the importance of exploring catalytic Ab as a potential SUD treatment, but it does not provide a detailed review.

A recent study reported the development of a novel biological agent against cocaine that incorporates both the long half-life of an Ab and the high catalytic activity of a cocaine

hydrolase (CocH).[168] The Ab-enzyme hybrid was constructed by replacing each variable (Fab) region of a human IgG_1 with a CocH, and by retaining the Ab constant region (Fc). The dimeric CocH-Fc fusion enzyme displayed high efficiency for cocaine hydrolysis, and Fc portion-dependent prolonged half-life.[168] This study shows how the field of biologics for SUD is constantly evolving.

Gene therapy

To improve passive immunization efficacy, and circumvent some of the limitations of mAb-based therapy, gene therapy has been explored as a new strategy to deliver anti-drug mAb.[169,170] This approach made use of adeno-associated viral-mediated transfer of genes encoding full anti-nicotine and anti-cocaine mAb, and showed efficacy in various pre-clinical models.[169,170]

Gene transfer strategies have also been examined to prolong, or enhance, the effect of cocaine hydrolases.[12] Viral vector-mediated gene transfer of a BChE-based mutant engineered for high catalytic activity, showed pre-clinical efficacy alone, or in combination with a cocaine vaccine, by blocking cocaine-induced motor activity in mice and rats challenged with doses up to 120 mg/kg.[171] Additionally, helper-dependent adenoviral vector-mediated delivery of cocaine hydrolases showed long-term efficacy in blocking cocaine self-administration in rats.[172]

To facilitate translation and regulatory approval, gene therapy approaches for treatment of SUD will need to be carefully evaluated for host immune responses to the viral vector components and other potential adverse effects in chronic toxicology studies. For instance, viral-mediated transfer of BChE did not show signs of motor, cognitive, or cardiovascular toxicity in mice and rats.[172-175] Despite promising pre-clinical proof of efficacy and safety, it is not yet clear whether gene transfer approaches will be acceptable by the FDA, EMA, or other regulatory agencies. To date, the FDA's Center for Biologics Evaluation and Research (CBER) has not yet approved any human gene therapy product for market.

Biologics for SUD: Economic incentives for translation

Medication development is a costly and laborious process. The potential revenue from a biologic for SUD could be roughly estimated from sales data of current medications for SUD.[8] Varenicline (Chantix®, Pfizer) made $647 million in worldwide sales last year, of which $377 million was made in the U.S. Depot naltrexone (Vivitrol®, Alkermes) made a total net sale of $94.2 millions in 2014 in the U.S. (www.alkermes.com). Sublingual buprenorphine/naloxone (Suboxone®, Reckitt Benckiser Pharmaceuticals) reached total net sales of $7 million in 2015, ranking 17th among the 100 best-selling, most prescribed branded drugs in the U.S. between April 2014-March 2015 (www.medscape.com). These data support development of biologics for SUD and steps toward technology commercialization through academic entrepreneurial activities, academia-private sector collaborations, NIH-funded small-business innovation research (SBIR) awards, attracting private investors, or licensing to pharmaceutical companies.

Conclusions

Vaccines and other biologics offer promising strategies to treat SUD. Pre-clinical and clinical studies will benefit from a better understanding of the cellular and molecular mechanisms underlying activation of specific B and T cell lymphocyte populations involved in generation of effective polyclonal anti-drug Ab responses. These data will provide a blueprint for rational development of more effective next-generation vaccines for SUD. B or T cell-based blood biomarkers predictive of vaccine efficacy will likely support patient stratification in clinical trials and identify patient subsets that will benefit from vaccination, or other therapeutic options. Development of newer hapten synthesis strategies, use of more immunogenic and well characterized carriers, better adjuvants, modular or particle scaffolds, and cutting-edge biomaterials will generate more effective next-generation vaccines for SUD. In addition to vaccines, other biologics such as anti-drug mAb and drug-degrading enzymes have shown promising pre-clinical and clinical outcomes. Once approved, biologics will expand the arsenal of therapeutic options to treat SUD. In the clinic, vaccines, anti-drug mAb and enzymes, and/or medications may be administered as combination therapy to circumvent the shortcomings of each individual line of treatment or as individualized therapy to target specific patient populations.

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