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Selected approaches for increasing HIV DNA vaccine immunogenicity *in vivo*

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Abstract

The safety, stability, and ability for repeat homologous vaccination makes the DNA vaccine platform an excellent candidate for an effective HIV-1 vaccine. However, the immunogenicity of early DNA vaccines did not translate from small animal models into larger non-human primates and was markedly lower than viral vectors. In addition to improvements to the DNA vector itself, delivery with electroporation, the inclusion of molecular adjuvants, and heterologous prime-boost strategies have dramatically improved the immunogenicity of DNA vaccines for HIV and currently makes them a leading platform with many areas warranting further research and clinical development.

Introduction

Owing to recent technical improvements, which have shown improved immune potency *in vivo*, plasmid DNA vaccines are again gaining importance as parts of potential HIV-vaccine platforms. DNA vaccines have an impeccable safety profile, are relatively easy to manufacture and to manipulate, and are remarkably stable making for ideal distribution in developing nations. Despite promising initial studies in small animal models [1–4], immunogenicity in larger species including non-human primates (NHP) and humans was disappointing [5,6]. Following up on these early studies, improvements to the vectors, insert sequence optimization, novel delivery methods and the inclusion of gene adjuvants has greatly improved both cellular and humoral immune responses induced by this platform so that HIV DNA vaccines are now performing as well as highly immunogenic viral vectors [7••]. These improvements make DNA vaccines more attractive as components of prime-boost platforms as well as in particular stand alone situations. This brief review will illustrate some important ways that have served to improve the immunogenicity of DNA vaccines to HIV-1 such as improved delivery, the inclusion of molecular adjuvants, as well as prime-boosting.

Delivery

An important approach to improve DNA vaccine immunogenicity is to increase the amount of DNA/plasmid delivered to cells, as this increases antigen expression. Various plasmid

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needle-free delivery devices have been studied in this regard, such as the gene gun, patch systems or jet injection devices. Jet injection involves using high pressure to deliver a liquid formulation of DNA mm to cm below the skin's surface. It has been reported effective in delivery of DNA through the skin deep into the muscle below and has been studied in the clinic. One issue with the performance of jet injection is a limitation on the volume of liquid that can be loaded into the delivery system, which is usually 1 ml. Accordingly, formulations require high concentrations of DNA. This technique is very adaptable and more studies are in progress. A more superficial delivery system is DNA administration by gene gun. This platform utilizes high pressure to deliver DNA coated gold nanoparticles to the dermis, an antigen presenting cell rich area. This approach has been particularly effective at driving antibody production *in vivo*. Only a small amount of DNA is needed for this approach and seroconversion can be observed with very low doses of DNA delivered in diverse animal systems as well as humans, resulting in very encouraging results. There are limitations in scaling up delivery with this platform, as the amount of gold beads that can be loaded with DNA and delivered at a single time is limited, thus effectively reducing the volume that can be delivered. More recently needle-free delivery as well as patch systems has been studied successfully in smaller animal systems. These are desirable vaccine delivery approaches and while these technologies are early in their study they will continue to receive attention in the future [8,9].

An older technology that has been reinvented and reapplied with vigor the DNA delivery area is *in vivo* electroporation (EP). EP has been used to transfect cells *in vitro* for almost 30 years. Recently it has been explored to increase the transfection efficiency of DNA vaccines *in vivo* [10]. Electroporation involves applying a small electric field across the site of injection in order to cause temporary membrane instability and produce an electric gradient, which increases cellular uptake of locally delivered macromolecules such as DNA [11,12]. Intramuscular vaccination has been extensively studied in NHP and safely used in humans. Initial studies with an HIV-1 DNA vaccine in rhesus macaques demonstrated that delivery with EP resulted in 10–40 fold increases in HIV-specific ELISpot responses compared with a fivefold higher dose of naked DNA [13••]. *In vivo*, EP has also been shown to increase the quality of the HIV-specific immune responses by increasing $CD8^+$ T-cell proliferation and functionality compared with naked HIV-DNA vaccination alone [14]. Though DNA vaccines were traditionally largely considered a platform for induction of cellular immunity, delivery with EP generates robust humoral immunity to SIV as well [15]. DNA vaccines to influenza virus delivered by EP induce titers of 1:40 tested by a hemagglutination inhibition assays in ferrets, and thus the benchmark for product development against this pathogen [16]. The safety and immunogenicity of a HIV DNA vaccine delivered with IM EP has recently been evaluated in phase I clinical trials (HVTN 080) and preliminary reports demonstrate enhanced cellular immunity compared with IM DNA delivery without EP.

Though IM EP has been extensively studied and results in consistently and reproducibly enhanced immune responses, many variations on this theme are in development. The field is focusing on alternative targets for DNA delivery by EP. Current research has led to new devices capable of highly efficient delivery to intradermal tissues for example. Furthermore, microneedles and needle-free electroporation arrays are being studied [17,18]. In addition to being easier to administer, dermal delivery may increase or uniquely modulate immunogenicity by directly transfecting APCs, which exist at high density in the skin [19]. Delivery of a DNA vaccine with a minimally invasive, low voltage EP device has been reported to induce protective immunity against an influenza challenge [20]. Newer devices do not generate tissue damage during routine vaccine delivery. How these new devices will impact current HIV DNA vaccines still needs to be evaluated in NHPs and humans but these approaches represent a straightforward improvement in vaccine delivery.

Molecular adjuvants

Molecular adjuvants are commonly used to increase or modify the immunogenicity of DNA-based vaccines. Molecular adjuvants can be divided into two groups: cytokine and chemokine molecules. Unlike traditional adjuvants, molecular adjuvants are delivered as plasmid vector along with the antigen-encoded vector. Upon delivery, the vector encoding the molecular adjuvant transduces cells at the site of vaccination that can then secrete the adjuvant molecule.

Cytokine molecular adjuvants can be generally classified as either Th1 promoting or Th2 promoting. Th1 adjuvants include IL-2, IFN-γ, IL-12, and IL-15 and generally work to augment cellular immunity [21–23]. By contrast, Th2 cytokines such as granulocyte-macrophage cell stimulating factor (GM-CSF), IL-1, and IL-4 enhance humoral immunity [24–26]. Because the activation of the Th1 or Th2 pathway inhibits the activation of the alternate pathway, care has to be taken with molecular adjuvants to not overly skew the response towards either pathway and inhibit induction of potentially crucial effector mechanisms [27].

Research into molecular adjuvants began over 10 years ago. GM-CSF was one of the first adjuvants to clearly demonstrate that a cytokine vector could modulate vaccine induced immunity. In mice receiving a rabies DNA vaccine alone, or co-administered with a GM-CSF expressing plasmid, GM-CSF adjuvanted mice showed enhanced cytotoxic T-cell, Th1 T-helper cell, and binding antibody titers [28]. Similarly, HIV vaccination with GM-CSF increased antibody responses and lymphopro-liferation in mice [29]. Recently, inclusion of a GM-CSF expressing plasmid delivered with a DNA-prime that was followed by a Modified Vaccinia Ankara virus (MVA)-boost resulted in enhanced IgG, neutralization, and increased protection from a repeat low dose SIVsmE660 challenge in vaccinated rhesus macaques [30].

Like GM-CSF, IL-12 was another early molecular adjuvant and the first Th1 adjuvant studied. As a Th1 adjuvant IL-12 has had profound positive effects on vaccines tested in nonhuman primates. In mouse models, IL-12 increased $CD8^+$ T-cell mediated lysis of target cells 4.5 fold [31]. HIV-1 vaccination of non-human primates with IL-12 demonstrated increased cellular responses that corresponded with control of viremia and improved clinical outcomes following a SHIV98.6P challenge [32]. The ability of IL-12 as a genetic adjuvant to increase HIV-1-specific responses following HIV-1 DNA vaccination with gag, env and pol is currently being examined in the clinic through the HIV Vaccine Trials Network. These IL-12 adjuvanted DNA vaccines have been well tolerated and they were shown to be immunogenic in humans.

IL-15 alone and in combination with IL-12 has also been extensively studied as an adjuvant to DNA vaccination. Together with IL-7 and IL-2, IL-15 provides signals important for T-cell survival. Rhesus macaques vaccinated with an HIV DNA vaccine and IL-15 demonstrated increased levels of effector memory $CD8^+$ T-cells [33]. Unlike most Th1 adjuvants, IL-15 has also been shown to enhance DNA vaccine-induced humoral immunity [34]. The ability to augment both humoral and cellular immunity makes IL-15 an interesting adjuvant for HIV vaccine development, where efficacious vaccines need to induce both humoral and cellular immunity.

Chemokine adjuvants

Chemokines are molecules that control the trafficking of lymphocytes and direct them to sites of inflammation. By utilizing chemokine molecular adjuvants, it is possible to modulate the magnitude and characteristics of DNA vaccine-induced mucosal immunity.

This may be particularly important for an HIV vaccine that needs to induce mucosal protection to prevent infection. CCL27 (CTECK) has been shown to increase mucosal homing of antigen-specific cells in mice and monkeys following a HIV-1 DNA vaccination [35]. Though studies with mucosal chemokines are ongoing, these data support the concept that a HIV DNA vaccine delivered IM could induce mucosal immunity, which would be highly advantageous to ward off pathogens that invade through the airway, the genital, or the intestinal surfaces.

Prime-boosting

An effective HIV-1 vaccine will probably need to induce robust effector T-cell responses as well as broadly neutralizing and binding antibody responses. One method of improving the magnitude and quality of the overall HIV-specific response is the use of heterologous prime-boost strategies. By combining DNA vaccines with either viral vectors or a recombinant protein a synergistic enhancement of immunity can improve challenge outcome. This concept has received a significant boost in attention owing to the success in the RV-144 'Thai' HIV vaccine trail [36••]. After two clear failures in the attempt to show vaccine efficacy in clinical trials, RV-144 achieved a modest but important success. A prior trial of one of the RV-144 components, that is, Vaxgen recombinant protein immunization for induction of antibody responses was unsuccessful in preventing HIV infection. Similarly, vaccines designed to only induce T-cell responses such as a recombinant adenoviral vector of serotype 5 (Merck STEP trial), were also not effective at preventing HIV infection, or lowering viral load [37• •]. By contrast, the RV-144 trial used viral vector priming with a recombinant poxviral vector, followed by boosting with the Vaxgen recombinant envelope antigen. This trial showed partial effectiveness at preventing infection for up to 6 months post the full vaccine protocol. Table 1 provides some strengths and weaknesses of these major vaccine development platforms.

The inability to induce HIV-1-specific broadly neutralizing antibodies through traditional immunization strategies has led many in the field of HIV vaccine development to focus on the need for cellular immune responses capable of clearing virally infected cells. Among the most potent approaches for induction of a focused $CD8^+$ T cell response is DNA-prime followed by viral-vector-boost. This dual modality approach drives effective improvement in T cell expansion that allows partial bypass of anti-vector induced immunity. DNA vaccine priming has been shown to focus the immune response on the plasmid-encoded antigen. When followed by the viral vector boost, expansion of the immune response is now focused on the vector-encoded vaccine antigens, rather than the nonrelevant antigens that are part of the viral vector.

By contrast, viral vector priming results in significant anti-vector immunity that reduces the ability of further homologous vaccination to boost responses primed by the initial dose [38].

The most extensively studied viral vectors for an HIV heterologous prime-boost vaccine include those based on adenoviruses and poxviruses (Table 1). The replication defective adenovirus serotype 5 (Ad5) vector gained popularity owing to its ability to generate robust effector $CD8^+$ T-cell responses and impact viral load following a SHIV, SIVmac239 and SIVmac251 challenge when used alone or in combination with a DNA-prime [39•,40•,41•, 42•]. These vectors remain the most robust ever developed for driving a T cell response in humans. However, reduced efficacy and safety concerns pertaining to vaccinating subjects naturally seropositive to Ad5 lead to the development of alternative rAd vectors with a naturally lower seroprevalence than Ad5 [37••,43]. This is particularly important in the developing world vaccine setting, where Ad seropositivity is particularly common.

Several classes of rare Ad vectors have been explored recently. These include rare human serotypes such as Ad11, Ad35, and Ad26 that have a seroprevalence of 5–15% in developed countries compared with the 50–90% observed for Ad5 [44,45]. Another approach involves using rAd vectors based on chimpanzee Ads that have a seroprevelence of less than 10%, even in zoo workers and populations living in close proximity with wild chimpanzees [46]. Rare Ad vectors have the ability to induce robust HIV-specific cellular responses in NHP [47,48]. However, limited work has been done to determine whether an HIV DNA-prime can further increase efficacy. Additionally, owing to a 90% capsid sequence homology between all Ad serotypes, human trials will be crucial to determine the safety and efficacy of these vectors as even seronegative subjects have cross-reactive Ad-specific T-cells, which may react to Ad vector vaccination [49] and modulate vector driven immunity.

The development of modern EP technology has provided a new option for DNA-prime viral vector boosting. In the literature, most DNA prime-boost studies were performed with older DNA strategies that generated weak immunity when administered alone. We have previously shown that repeated immunization with a highly optimized SIV DNA with IL-12 and electroporation can induce cellular responses as good or better than rAd5 [7••]. By combining priming with a highly optimized DNA vaccine we were not only capable of generating robust T-cell responses [7••], but we also observed that a year after priming, these responses were efficiently boosted with only two doses of a highly immunogenic rAd5-SIV vaccine (Figure 1). These data warrant further studies to optimize prime-boost strategies utilizing newer, more robust HIV DNA vaccine platforms.

In addition to rAd vectors, extensive research has gone into the use of poxvirus vectors such as NYVAC, a highly attenuated vaccinia virus strain, canarypox, and MVA. Though comparative studies have suggested that Ad5 is more immunogenic than poxviral vectors [39•,41•] for driving cellular immune responses, studies in both NHP and humans demonstrated that a DNA-prime MVA-boost can generate enhanced antibody and cellular responses to HIV/SIV antigens [50].

Several versions of poxviral prime-boost studies are in development. One such study that has recently started is RV-262, which examines the ability of a highly optimized DNA vaccine delivered by EP to be boosted by a HIV recombinant poxviral vaccine. In this phase I study, called RV262, evaluation of a combination DNA-prime MVA vector-boost vaccine regimen is being studied. This approach seeks to drive immune responses against diverse subtypes of HIV-1 prevalent in North America, Europe, Africa and South America. The study is being conducted by the U.S. Military HIV Research Program (MHRP) through its clinical research network in the U.S., East Africa and Thailand. This clinical trial was designed to test a unique prime-boost preventive HIV vaccination strategy aimed at global coverage. The prime is a plasmid DNA vaccine, PENNVAX-G, and the boost is a virus vector vaccine, MVA-Chiang Mai Double Recombinant (MVA-CMDR). Together, the vaccines are designed to deliver a diverse mixture of antigens for HIV-1 subtypes A, B, C, D and E. The results of this study will have important implications for EP-DNA prime-MVA-boost approaches.

The success of preclinical studies evaluating a DNA-prime viral vector-boost has lead to the initiation of two large clinical trials to further access the protective efficacy of these strategies in humans. One of the most prominent trials, HVTN 505, is a phase II study enrolling 1350 subjects to test the safety and efficacy of a DNA-prime followed by an Ad5-boost vaccination strategy developed by the Vaccine Research Center (VRC). Another phase II study is being conducted to evaluate the efficacy of a DNA-prime followed by a MVA-boost (HVTN 205).

In addition to viral vector boosting, DNA priming can expand host B cell immunity, which can be further boosted by a recombinant protein. In addition to improving the magnitude of HIV-specific immune responses, heterologous prime-boost strategies afford the possibility to modify the 'quality' of the immune response. In a study comparing a DNA-prime recombinant protein-boost versus either platform alone, the inclusion of a DNA-prime enhanced the avidity and neutralization of HIV-specific antibodies [51]. Importantly, the success of this approach achieved in animal models has translated to humans with the first report of neutralizing antibodies against clade A–E primary isolates elicited by a multiclade DNA-prime recombinant protein-boost strategy HIV-1 vaccine [52•,53•].

Though these results are promising, they may be further improved by delivery of a DNA priming with EP. In a study comparing a HIV clade A and C DNA vaccine delivered with IM EP and followed by a gp120 boost either alone or in combination in BALB/c mice, we observed that three doses of DNA alone induced a higher binding antibody titers than a single dose of protein alone (Figure 2). However, a prime-boost strategy resulted in the most robust antibody titers. The promising results of these studies are being confirmed in rabbits and NHPs where not just binding, but neutralizing titers can be studied. Currently, the ability of a multivalent HIV DNA-prime, recombinant envelope protein-boost to enhance cellular immune responses and induce cross-subtype neutralizing antibodies is being evaluated in a phase I clinical trial (DP6-001) [53•].

Conclusions

Intensive research over the past 20 years has lead to remarkable improvements in the immunogenicity of HIV DNA vaccines. Following years of failures, the recent RV-144 trial, showing a modest prevention of heterosexual transmission, has been viewed as an important achievement that is refocusing efforts in the HIV vaccine field, and engendered a renewed interest in prime-boost strategies. Many clinical trials are ongoing and will provide important data for further development. New developments in DNA technologies are likely to play a more and more important role in future HIV vaccine studies. Further research into delivery, molecular adjuvants and prime-boost strategies may generate the magnitude, quality and breadth of cellular and humoral responses needed for an effective HIV vaccine.

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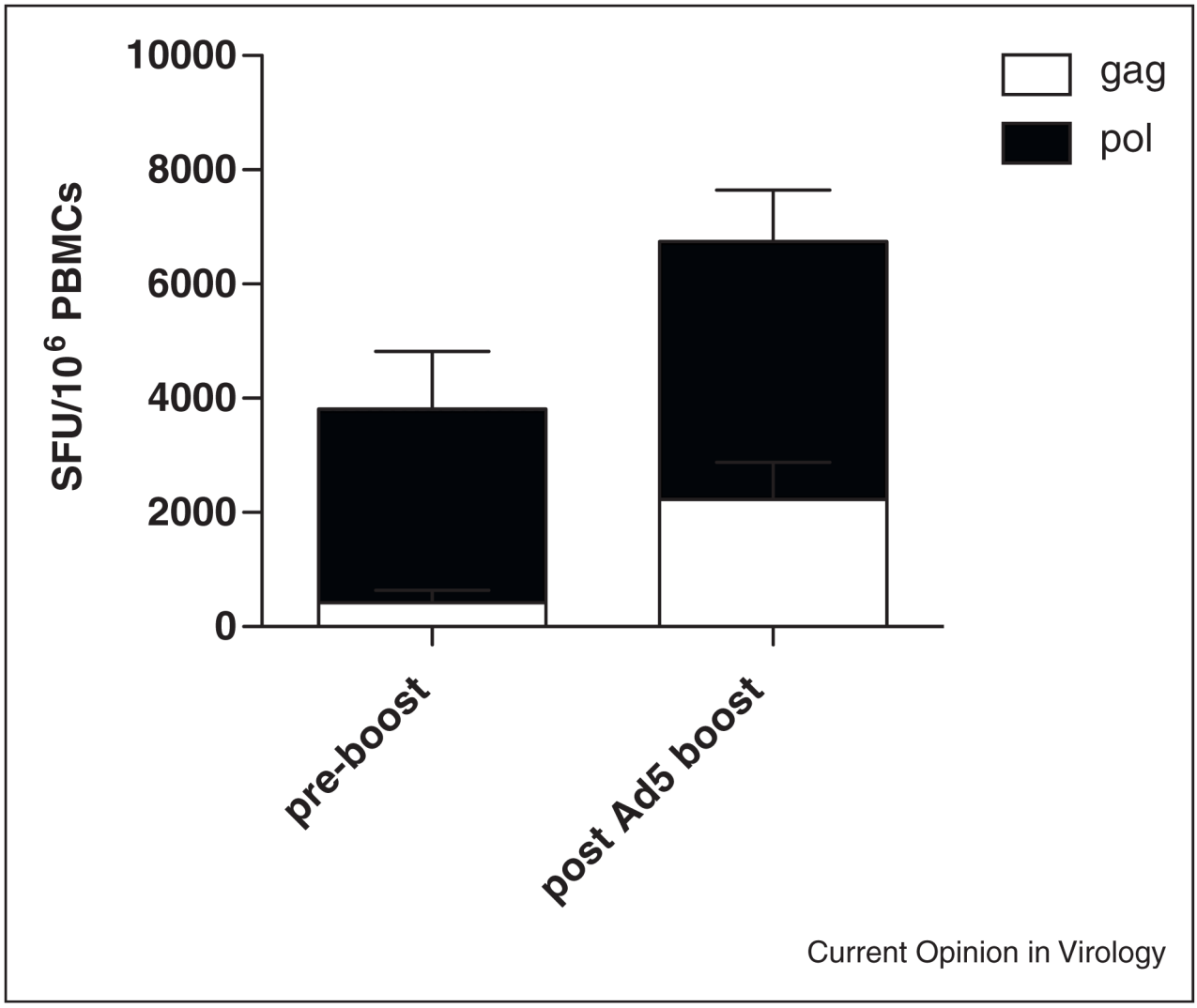

Figure 1.

An SIV DNA-prime Ad5-boost enhances cellular immunity. Five Indian rhesus macaques received three doses of 1.0 mg each pVax consensus SIV gag/pol/env at weeks 0, 6, 12, and 18 delivered intramuscular with electroporation (Inovio Pharmaceuticals, Inc.) followed by two doses of 1×10^{10} viral particles (vp) Ad5 SIV gag/pol/nef (Merck and Company) at weeks 52 and 56. Following two doses of an Ad5 boost, IFN-g responses were significantly increased from pre-boost levels ($p < 0.03$).

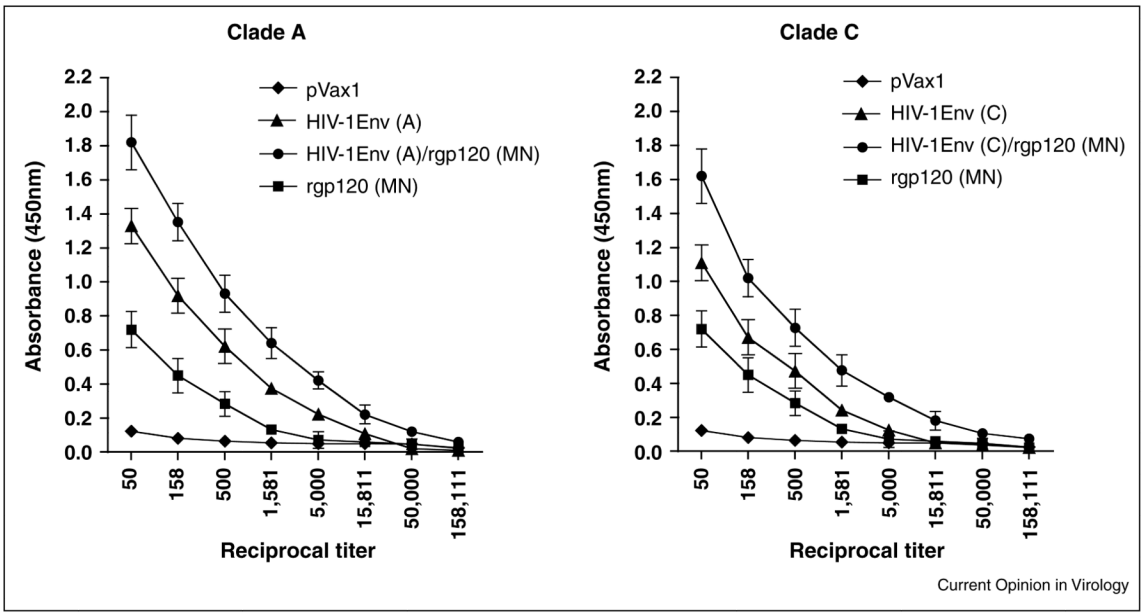

Figure 2.

Improvement of antigp120IgG responses after protein boosts in Balb/C mice primed with HIV-1 Envelope DNA vaccine. Four groups of 4 mice were immunized either three times with pVax DNA (diamond) or HIV-1Env DNA (triangle) in 2 weeks interval, three doses of HIV-1 env DNA followed by a protein boost (HIV-1Env/rgp120, circle), or a single dose of rgp120 (square). Sera were collected two weeks after the final vaccination. Binding of antisera against subtype A or C gp120s to envelope proteins from clade B viruses (MN). Values represent mean ($n = 4$) and the SEM.

Table 1

Comparison of some advantages/disadvantages of important vector vaccine platforms for generating HIV-specific cellular immunity

Vaccine platforms	Advantages	Disadvantages
DNA vectors	can be used for homologous boosting	Immune responses have historically been low – EP strategies look improved
	stable	Limited local site delivery of antigen
	generates cellular and humoral immunity	Delivery enhancement is important to overcome current limitations
	successful licensure of veterinary vaccines	Improved vaccine formulations important
Adenovirus vectors	robust CD8+ responses, combination serotypes and species unique viruses improve responses	pre-existing immunity in many populations limits effectiveness
	efficiently transduces mammalian cells	safety concerns in seropositive
	Most potent viral platform to drive cellular immunity in humans	need for complementing cell lines
poxvirus vectors	limited pre-existing immunity	anti-vector immunity
	successful licensure of veterinary vaccines	reduced immunogenicity compared with other viral vector platforms
	Promising results in RV-144 Thai trial	