Purpose of review
To discuss recent progress in the use of vectors to produce antibodies in vivo as an alternative form of HIV prophylaxis or therapy. Instead of passive transfer of monoclonal antibody proteins, a transgene encoding an antibody is delivered to cells by the vector, resulting in expression and secretion by the host cell. This review will emphasize adeno-associated virus (AAV)-based strategies and summarize the evidence in support of this strategy as an alternative to traditional vaccines. We will highlight the major findings in the field and discuss the impact that this approach could have on the prevention, treatment and possibly eradication of HIV in patients.

Recent findings
In this emerging field, the emphasis has been on the use of vectors delivering antibodies as an alternative to the development of an HIV vaccine. However, recent findings suggest that AAV-delivered broadly neutralizing antibodies can suppress HIV replication. As such, a single injection of AAV could mediate long-term antibody expression to act as a long-lived therapeutic in the absence of antiretroviral drugs.

Summary
Vector-mediated antibody expression can both prevent transmission and inhibit the replication of established HIV infections. As such, it offers an alternative to immunogen-based vaccine design and a novel therapeutic intervention by enabling precise manipulation of humoral immunity. Success may enable not only the development of effective prevention against HIV but may also provide an alternative to a lifetime of antiretroviral drugs taken by those who are already infected.

Keywords
antibody gene transfer, broadly neutralizing antibodies, HIV vaccine, immunotherapy, vectored immunoprophylaxis

INTRODUCTION
The isolation of broadly neutralizing antibodies (bNAbs) from patients capable of recognizing diverse strains of HIV-1 [1–11] has led to an intense investigation into their possible use as prophylaxis [12–17,18**] or immunotherapy [19**,20,21**,22*,23–25,26**,27,28]. These bNAbs typically arise within 2–4 years of infection in 10–30% of patients [29–31] and share a number of unusual features that make the prospects for eliciting similar antibodies by immunization uncertain [32]. This includes long CDRH3 regions that make important contacts with the virus [33,34] and very high numbers of somatic mutations, which arise in parallel with the sequence diversification of the initial infection [35,36]. Our present understanding of immunogenicity is insufficient to rationally design vaccine immunogens capable of eliciting bNAbs in human patients. Although a series of immunizations has been proposed as a means of guiding the immune system to develop bNAbs, these may be difficult to implement if numerous immunizations over long periods of time are required. Additionally, it is uncertain whether diverse human populations will respond to an immunogen-based vaccine regimen consistently enough to elicit bNAbs in the majority of recipients.

In contrast, passive transfer of bNAb proteins could circumvent all of these uncertainties to provide consistent protection in all recipients. However, the relatively short half-life of antibodies...
in vivo, which averages 21 days [37], means that such strategies would require regular readministration. Past clinical experience testing preexposure prophylaxis suggests that interventions requiring consistent readministration generally suffer from insufficient compliance to consistently yield protection [38]. Although Fc mutations that improve antibody affinity for the neonatal Fc receptor have been shown to increase the half-life of antibodies up to five-fold in vivo [39,40], the impact of these mutations on the long-term immunogenicity of antibodies bearing them in patients is unknown. Additionally, antibodies require temperature-controlled storage and distribution networks that are only available in well developed healthcare systems. Together these challenges make the widespread use of bNAb proteins by passive transfer for the prevention or treatment of HIV infeasible, particularly in the developing world where the need is greatest.

**KEY POINTS**

- Vectored antibody delivery represents a new strategy for the production of bNabs in vivo.
- bNabs have been shown to prevent HIV transmission in numerous animal models.
- Vectored antibody delivery has the potential to be used as a long-lived therapeutic intervention for HIV-infected patients.
- Two clinical trials are ongoing or planned to determine the safety of vectored antibody expression in both uninfected and infected patients.

**VECTORED ANTIBODY GENE DELIVERY**

Several groups have put forth alternative strategies based on gene transfer to enable the production of bNabs in vivo. These approaches deliver transgenes encoding antibodies to tissues using a variety of vector platforms to express and secrete antibody. As such, these strategies leapfrog traditional concepts of humoral immunity and altogether bypass the adaptive immune response to enable the production of desirable antibody specificities without the use of immunization or passive transfer (Fig. 1). Several vectored antibody strategies have been investigated as an alternative form of prophylaxis against HIV.

Nonviral vectors, such as naked DNA, offer simplicity of design, ease of manufacturing and relatively low immunogenicity. Recently, optimization of the expression plasmid and electroporation conditions for muscle targeted delivery has yielded production of up to 2–3 μg/ml of the VRC01 heavy and light chain (i.e., Fab fragments) in plasma for 12 days following administration [41*]. Although an important proof-of-principal for rapid delivery, the modest concentrations and transient nature of expression from current plasmid transfection approaches diminish the potential for such strategies to function as a long-lived prophylaxis.

In contrast, lentiviral vectors represent an attractive means of integrating foreign genes into the host genome, allowing for stable transgene expression that is inherited by daughter cells. Most lentiviral vectors are derived from the HIV-1 genome, and are pseudotyped with VSV glycoprotein, expanding vector tropism to include most types of dividing and nondividing mammalian cells [42]. Primary human hematopoietic stem cells...
(HSCs) have been transduced with lentiviral vectors encoding the heavy and light chains of the b12 bNAb, resulting in the production of plasmablasts that secrete b12 in vitro [43]. A similar approach was used to engineer B cells to secrete the 2G12 bNAb in humanized mice [44]. Although these B cells did not express surface 2G12, and thus would not proliferate following antigenic stimulation, the concentration of secreted 2G12, approximately 40 ng/ml, was sufficient to inhibit HIV infection in vivo [44]. Similar studies produced BLT mice harboring engineered HSCs to express an immunoglobulin A form of the b12 antibody, which resulted in protection of mucosal CD4+ cells following intravaginal challenge [45]. Whereas these studies demonstrate exciting proof-of-principle for lentiviral vectors to genetically engineer HSCs to secrete bNAbs, transduction was performed ex vivo, a scenario that is unlikely to be feasible on a large scale or in low-resource settings.

Recombinant adeno-associated virus (rAAV) is perhaps the best studied vector for gene transduction in humans, with over 100 clinical trials demonstrating safety of transduction [46]. It was recently approved in Europe as the first gene therapy product for use in humans to treat lipoprotein lipase-deficiency [47], and exciting results have been reported using AAV for the treatment of hemophilia [48*,49]. AAV is a parvovirus that packages a single-stranded DNA genome and exhibits distinct cellular tropism that is primarily defined by the viral capsid [50]. Recombinant AAV can be produced using the inverted terminal repeat sequence elements from the AAV2 serotype, packaged with homologous capsids from other serotypes [51]. Although natural AAV infection is nearly ubiquitous, with approximately 80% of humans seropositive for the AAV1 or AAV2 capsid [52], it is not associated with any known disease [53]. Given that preexisting immunity severely limits the efficiency of transduction [54], considerable effort has been expended identifying novel capsids from other species [55] and generating entirely new capsids through structural engineering and selection [56,57].

Despite numerous advantages, AAV is limited to packaging genomes smaller than about 5 kb of single-stranded DNA [58,59]. This packaging limitation is even more severe in so-called self-complementary AAV (scAAV), which packages a double-stranded DNA genome at the expense of half of the packaging capacity, but which exhibits more efficient expression than natural AAV [60,61]. The packaging limitation of AAV represents a significant challenge for the efficient delivery of both heavy and light chains that form the natural antibody structure, stimulating the development of a number of alternative strategies to circumvent this limited space.

**ANTIBODY TRANSGENES FOR ADENO-ASSOCIATED VIRUS-VECTORED EXPRESSION**

Lewis et al. [14] first described the delivery of antibodies with AAV by constructing a dual-promoter vector, whereby the heavy and light chain genes of the b12 bNAb were independently transcribed from separate promoters. Following a single intramuscular injection of recombinant AAV1, immunodeficient Rag mice expressed up to 8 μg/ml of biologically active human immunoglobulin G1 (IgG1) in circulation for over 6 months (Fig. 2a) [14]. However, highly efficient expression of full-length antibodies was first achieved by Fang et al. [62] who used the foot-and-mouth disease virus-derived 2A self-processing sequence (F2A) to express both heavy and light chain genes from a single open reading frame. Careful placement of the F2A sequence adjacent to a modified furin cleavage site resulted in expression of fully assembled antibody indistinguishable from the natural protein by mass spectroscopy at sustained serum concentrations above 1000 μg/ml in vivo [62,63].

The limited carrying capacity of scAAV vectors necessitated the use of alternative antibody architectures that could be encoded in this space. Immunoadhesin molecules consisting of single-chain Fv domains attached to natural Fc-region via artificial serine–glycine linkers have been shown to maintain epitope recognition as well as a long half-life [64]. However, careful characterization of such immunoadhesins is necessary as some single-chain Fv proteins exhibit reduced neutralization potency as compared with the parent IgG, likely due to a reduced affinity for the antigen-binding site [65]. As initial experiments in macaques using the previously characterized rAAV-IgG1 b12 vector [14] resulted in the loss of antibody expression due to a strong antihuman transgene immune response, simian immunodeficiency virus (SIV) gp120-specific immunoadhesins were explored as an alternative to full length antibodies that could be delivered by scAAV1 (Fig. 2b) [13]. Following administration of $2 \times 10^{13}$ genome copies of vector, immunoadhesin expression peaked at a concentration of approximately 200 μg/ml at 3–4 weeks post injection and was sustained at 20 μg/ml for the past 4 years, demonstrating significant long-term expression [66]. Six of the nine monkeys challenged intravenously with 40 macaque infectious doses (143 ng of p27) of SIVmac316 molecular clone a month after rAAV administration were completely protected from
challenge as determined by a lack of plasma SIV RNA for over 6 years [13,66]. Of the three immunized macaques that became infected after challenge, all had developed a significant immune response to the immunoadhesin 1 week before challenge, suggesting that the presence of anti-immuno-adhesin antibodies were responsible for the failure of protection [13].

We have previously described the development of a vector capable of eliciting long-lived expression of high concentrations of full-length human bNAbs from muscle in mice using an optimized transgene packaged with AAV serotype 8 (Fig. 2c) [12]. This capsid, originally isolated from rhesus monkey, has shown a propensity for transducing muscle cells [55] and has been associated with reduced immunogenicity in vivo [67]. A single intramuscular injection of $1 \times 10^{11}$ genome copies of this vector into humanized mice resulted in the production of antibody at circulating concentrations greater than 100 μg/ml, which persisted for at least 52 weeks [12]. This approach, coined ‘vectored immunoprophylaxis’ (VIP), demonstrated robust protection against intravenous HIV challenge with at least 125 humanized mouse infectious doses (125ng of p 24) of HIV molecular clone NL4–3 [12]. By administering decreasing doses of vector, we determined that protection against intravenous challenge was clearly dose dependent, requiring a minimum of 34 μg/ml of b12 or 8.3 μg/ml of VRC01 [12]. In recent studies with more potent bNAbs, we have reported protection from the same intravenous challenge with as little as 0.35 μg/ml of VRC-PG04 [18].

Whereas we found that VIP was highly efficacious against intravenous HIV transmission, most new infections worldwide are the result of sexual intercourse. As it was unknown whether VIP would also be effective against HIV transmission across the vaginal mucosal surface, we developed a repetitive, nonabrasive, low-dose challenge model in bone marrow–liver–thymus (BLT) humanized mice to better mimic human exposure. BLT mice offer an advantage over other humanized mouse models in that they exhibit stable multilineage hematopoiesis and T-cell education in the human thymus graft [68,69]. Additionally, these mice exhibit extensive engraftment of immune cells throughout mucosal tissues, enabling the study of HIV transmission across mucosal surfaces [70]. In our studies, 63% of mice transduced with AAV-VRC01 were protected from 15 consecutive weekly intravaginal challenges with a CCR5-tropic molecular clone of HIV (JR-CSF). The three animals expressing VRC01 that were not protected became infected only after 13–15 consecutive exposures as compared with an average of 4–5 exposures for control mice [18]. In addition, challenge experiments performed using a transmitted founder strain of HIV (REJO.c) in animals expressing the VRC07G54W bNAb achieved complete protection despite 21 consecutive weekly vaginal exposures, suggesting that VIP could result in effective prevention against mucosal transmission of strains that have previously transmitted between humans [18]. We believe the efficacy achieved in these studies may represent an underestimation as even our ‘low dose’ exposures resulted in
transmission within 4–7 challenges. In contrast, heterosexual human transmission has been estimated to occur between 1 in 100 and 1 in 1000 exposures [71,72].

**VECTORED ANTIBODY DELIVERY AS A LONG-LIVED HIV IMMUNOTHERAPEUTIC**

Although it is clear that bNAbs are capable of preventing HIV transmission in animal models, there is exciting evidence from passive transfer experiments suggesting that bNAbs have the potential to control established HIV replication and perhaps even impact the viral reservoir [22*,26**]. Passive immunotherapy with first-generation bNAbs in humans resulted in viral escape during treatment [23,24]. More recent studies utilizing cocktails of newly described bNAbs have resulted in a transient decrease in viremia of HIV-infected humanized mice [28] and simian/human immunodeficiency virus (SHIV)-infected rhesus macaques [20,21*,22*]. Most of these studies found that viral escape occurred after passive transfer of individual bNAbs [21*,23,24,28,73] but that a cocktail with as few as two bNAbs could maintain viral suppression [28]. However, a recent study by Horwitz et al. [19**] offers tantalizing evidence that even a single bNAb delivered by AAV may be capable of maintaining the reduction of previously suppressed viral replication. HIV-infected humanized Rag mice were treated with antiretroviral therapy (ART) for 5 days followed by a combination of ART and bNAb 10–1074 protein for 16 days prior to cessation of drug treatment. Passive transfer with 10–1074 was continued for 12 more days after which mice were given 2.5 × 10**11** genome copies of AAV-10–1074, resulting in prolonged control of HIV infection after ART was removed [19**]. Horwitz et al. [19**] hypothesize that viremia must first be controlled with ART as AAV vectors express too slowly to work therapeutically on their own. The combined use of ART and vectored-antibody gene delivery may be difficult to implement in humans as the potential impact of antiviral HIV drugs on AAV transduction has not been described in the literature. If, for example, existing nucleoside analogs used in the treatment of HIV interfere with the natural conversion of single-stranded AAV to the transcriptionally active double-stranded form, novel AAV-compatible HAART regimens will need to be developed to implement this approach in patients. Alternatively, the rapidly expanding constellation of ever more potent bNAbs may enable control of HIV by AAV-mediated antibody delivery without the need for prior viral suppression. A more thorough understanding of the evolutionary dynamics of viruses undergoing suppression by bNAbs may enable the development of new antibody regimens capable of consistently control HIV replication.

Whereas permanent bNAb-mediated suppression of HIV may one day result in a ‘functional’ cure of HIV infection, the persistence of the latent viral reservoir complicates efforts to achieve complete eradication. However, recent evidence suggests that antibodies may be capable of directing immunological responses against the reservoir by targeting cells for destruction. Studies in humanized mice have demonstrated that antibodies can impact the viral reservoir when conjugated to immunotoxins [74**] or in conjunction with inducing agents [26**] to stimulate viral transcription. These combinatorial strategies to attack infected cells could represent a first step toward eradicating HIV infection in humans using antibodies.

**SAFETY OF CONSTITUTIVE EXPRESSION OF BROADLY NEUTRALIZING ANTIBODIES**

Although vectored-antibody gene delivery holds tremendous promise for the prevention and treatment of HIV, several safety concerns remain. HIV bNAbs exhibit an unusually high rate of somatic hypermutation with frequencies of up to 32% in the heavy chain variable region [7,8]. In contrast, the number of mutations observed in mature influenza antibodies rarely exceeds 10% [75]. It is unknown whether such highly mutated regions could make them targets of anti-idiotype responses, which may diminish their protective activity, lead to the loss of transgene expression [76], or result in toxicity stemming from immune complex deposition in the kidneys [77]. Additionally, some bNAbs are polyreactive and bind avidly to human antigens [78,79], creating the potential for autoimmune. No significant side-effects have been observed with bNAbs that have already been administered to HIV-1-infected patients (Table 1) [23,24]; however, it is uncertain whether this will remain true of more recently described antibodies. Many existing monoclonal antibody-based drugs administered clinically exhibit low but detectable levels of immunogenicity [37,86]. Over 30% of patients experience anti-idiotype responses when receiving antibodies originally isolated in mice that have been humanized, but this has fallen to fewer than 15% for antibodies derived from mice transgenic for the human immunoglobulin loci [87,88]. It is tempting to speculate that this rate may be further diminished for antibodies that were identified in humans and whose B-cells underwent immunological selection in humans. Given these uncertainties, it will be prudent to confirm the safety of bNAbs in the context of short-lived passive transfer of protein before vectored antibody delivery is attempted.
CONCLUSION

Vectored antibody gene delivery is a promising strategy for the precise manipulation of humoral immunity. In multiple animal models, it has enabled the production of well characterized, protective antibody responses that prevent transmission of HIV and other model pathogens. If similar results are obtained in human patients, VIP stands to sidestep existing barriers facing the design of an effective vaccine by offering an alternative to the use of immunogens to elicit protective antibodies. Several ongoing and planned clinical trials over the next several years are expected to address the safety and efficacy of bNAb in humans (Table 1). Results from these passive transfer trials will be instrumental for the studies testing AAV as a platform for the delivery of these antibodies in patients. Two such AAV trials have been planned: the first, sponsored by the International AIDS Vaccine Initiative is currently recruiting adult healthy men in the United Kingdom to receive AAV1 expressing PG9 bNAb. A second trial planned by the NIH Vaccine Research Center testing AAV8 expressing VRC07 in HIV infected patients is slated to begin enrolling participants in the next year. Results of these trials will go a long way toward revealing the challenges that will need to be solved to enable the widespread use of VIP for humans.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
■ of special interest
★★ of outstanding interest

This study is the first demonstration that vectored delivery of bNAbs can prevent with a combination of viral inducing drugs could impact the viral reservoir in This study is the first to demonstrate that passive transfer of bNAbs in conjunction This study demonstrated that 3BNC117 or 10-1074bNAbs could control viremia This study demonstrated the potential for PGT121, 3BNC117 and b12 to **suppress SHIV-SF162P3 viremia in macaques. It also showed that a macaque This study demonstrated that 3BNC117 or 10-1074bNAbs could control viremia This study is the first to demonstrate that vectored delivery of bNAbs can prevent intravaginal HIV transmission. It is also the first to study the use of transmitted molecular founder strains of HIV in a humanized mouse model. 19. Horwitz JA, Halper-Stromberg A, Mouquet H, et al. HIV-1 suppression and **neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A 2012; 109:18921–18925. 20. Peeling RW, Yang ZY, Boyington JC, et al. Neutralizing HIV-1 antibodies to herpes- SHIV vaccine virus. J Virol 2004; 78:8769–8775. 21. Lewis AD, Chen R, Montefiori DC, et al. Rational design of envelope identifies broadly neutralizing antibodies in humanized mice. Nature 2012; 492:118–129. 22. Balazs AB, Lu C-L, Klein F, et al. Enhanced muscle electroporation strategy to rapidly generate Fab fragments from B cells. Mol Ther 2012; 20:1831–1835. 23. Van der Straten A, Van Damme L, Haberer JE, Bangsberg DR. Unraveling the **antiretroviral therapy through passive transfer of human neutralizing antibo- **combines with a single vectored bNAb is capable of durably suppressing viral replication in humanized mice. 25. Wang L, Croce L, Bell P, et al. Impact of preexisting immunity on gene transfer to nonhuman primate liver with adenovirus vector B vaccines. Hum Gene Ther 2011; 22:1389–1401.


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