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REVIEW

Promise and problems associated with the use of recombinant AAV for the delivery of anti-HIV antibodies

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Attempts to elicit antibodies with potent neutralizing activity against a broad range of human immunodeficiency virus (HIV) isolates have so far proven unsuccessful. Long-term delivery of monoclonal antibodies (mAbs) with such activity is a creative alternative that circumvents the need for an immune response and has the potential for creating a long-lasting sterilizing barrier against HIV. This approach is made possible by an incredible array of potent broadly neutralizing antibodies (bnAbs) that have been identified over the last several years. Recombinant adeno-associated virus (rAAV) vectors are ideally suited for long-term delivery for a variety of reasons. The only products made from rAAV are derived from the transgenes that are put into it; as long as those products are not viewed as foreign, expression from muscle tissue may continue for decades. Thus, use of rAAV to achieve long-term delivery of anti-HIV mAbs with potent neutralizing activity against a broad range of HIV-1 isolates is emerging as a promising concept for the prevention or treatment of HIV-1 infection in humans. Experiments in mice and monkeys that have demonstrated protective efficacy against AIDS virus infection have raised hopes for the promise of this approach. However, all published experiments in monkeys have encountered unwanted immune responses to the AAV-delivered antibody, and these immune responses appear to limit the levels of delivered antibody that can be achieved. In this review, we highlight the promise of rAAV-mediated antibody delivery for the prevention or treatment of HIV infection in humans, but we also discuss the obstacles that will need to be understood and solved in order for the promise of this approach to be realized.

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Since the first reported cases of acquired immunodeficiency syndrome (AIDS) in 1981 (ref. 1) and the identification of the AIDS-causing virus in 1983 (ref. 2), it is estimated that more than 40 million people have died from human immunodeficiency virus (HIV) infection.[3,4] About 35 years have elapsed since the first documented HIV-1 infections and no substantial progress has been made in developing a vaccine that could effectively protect against HIV infection in the vast majority of people.[5–8] Similarly, with the single exception of the "Berlin patient",[9–11] eradication of HIV from infected individuals has also not been achievable.[12] Although the development of potent antiretroviral drugs has made it possible to vastly extend the life expectancy of HIV-infected individuals, anti-HIV drugs do not cure virus infection.[12–20] As of 2014, it was estimated that almost 37 million people were living with HIV globally, with a continuing new infection rate of 2 million per year.[21]

There are good reasons for believing that development of an effective vaccine against HIV-1 is going to be a very difficult task.[22,23] The predicted difficulties have more or less been borne out by vaccine trials in monkeys and in humans.[6–8,24] Of the six large-scale, placebo-controlled human efficacy trials of HIV vaccines, three showed no protection against acquisition and two actually showed enhanced acquisition of HIV-1 infection in the vaccine recipient.[25–37] Only one of the six vaccine trials, termed RV144 (ref. 38), appeared to show some protective effects against acquisition,[39–47] but claims regarding vaccine efficacy have not been straightforward to interpret. Furthermore, none of the six HIV efficacy trials reported a reduction of viral loads in vaccine recipients that became infected.

While attempts to develop improved vaccine strategies continue, many feel that alternate approaches that differ from conventional vaccination may be needed. One such alternate approach is the delivery of anti-HIV monoclonal antibodies (mAbs) by recombinant AAV (rAAV) gene transfer. This technology is independent of the host immune system and AAV-delivered antibodies have the potential to create a long-term sterilizing barrier against HIV. Studies that have employed rAAV vectors to deliver antibodies or antibody-like molecules have shown protective effects against simian immunodeficiency virus (SIV) in monkeys,[48,49] simian-human immunodeficiency virus (SHIV) in monkeys[50,51] and HIV in humanized mice.[52] Although encouraging, efficacy in monkeys was limited by immune responses to the delivered transgene product.[48,49,51] AAV-mediated delivery of broadly neutralizing antibodies (bnAbs) also shows promise for inhibiting viral replication and possibly even eradicating infection in HIV-positive individuals. Passive transfer of bnAbs to HIV-infected mice,[53–55] SHIV-infected monkeys,[56–58] and HIV-infected

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humans[59,60] has already shown potent antiviral effects when used as a therapeutic modality. However, those inhibitory effects against virus infection were transient due to the limited bioavailability of therapeutic antibodies following passive transfer. Recombinant AAV-antibody gene transfer could eliminate the need of repeated mAb infusions to already-infected humans and create constant, long-term levels of potent bnAbs in serum.

This review discusses the promise of AAV-delivered bnAbs for the ability to protect against the acquisition of HIV infection in humans and to block virus replication in those individuals that are already infected. We also discuss the problem of immunogenicity of AAV-delivered antibodies, which appears to be a major stumbling block to effective application of this approach for use in people.

THE ELUSIVE HIV VACCINE

Soon after HIV was discovered, the scientific community was optimistic that a vaccine against the AIDS-causing virus could be developed in a timely manner. That belief has unfortunately been shattered. More than 30 years of research have shown that a vaccine against HIV will be much more difficult to develop than the successful vaccines that exist for other pathogens.[5,22,61,62] The biggest challenge in the development of an effective HIV vaccine lies in the nature of the virus itself. HIV establishes a continuous presence by the integration of its genetic information into the host genome; it is able to generate and tolerate an enormous degree of genetic variation; and it has evolved a variety of strategies for evading host immune responses.[7,63–70] Once HIV establishes the initial infection, it is able to replicate continuously and without relent despite apparently strong humoral and cellular immune responses.[22] Factors that contribute to a failed immune control of HIV infection are summarized in (Figure 1).[23,69,71–78]

Since the first HIV vaccine trial in 1987 (ref. 79), more than 270 trials have followed.[80] From these, several vaccine candidates have progressed to a total of six phase IIb or phase III efficacy trials (Table 1).[6,7,81] AIDSVAX B/B used in VAX004 (refs. 25–28) and AIDSVAX B/E used in VAX003 (ref. 29) were the first HIV vaccines to enter phase III clinical trials. The vaccine preparations consisted of combinations of HIV recombinant gp120 envelope (env) proteins. As the name implies, AIDSVAX B/B included envelope protein sequences of two clade B isolates (MN, tissue culture derived strain; GNE-8, primary isolate). AIDSVAX B/E included the sequence of a clade B isolate (MN) and the sequence of a clade E isolate (CM244, primary isolate). The goal of the two studies was to test whether the gp120-induced antibodies were capable of preventing acquisition of the virus in high-risk populations. The outcome of the trials showed that the vaccines were not effective at preventing HIV infection; the rates of infection in the vaccinated groups versus the unvaccinated groups were similar. Furthermore, the vaccines had no influence on viral loads, CD4+ T cell counts or progression to AIDS.[25–29]

These first vaccine efficacy trials were followed by two very different efficacy trials that were based on viral vectors aimed at eliciting cellular responses against HIV. Virus vectors derived from replication-defective adenovirus serotype 5 (Ad5) were utilized in the STEP study[30–34] and the Phambili study,[35,36] numbers 3 and 4 of the HIV efficacy trials. The STEP study enrolled HIV-1-negative individuals that were given either a placebo or an equal mixture of three separate recombinant Ad5 vectors from the company Merck (MRKAd5). The three vectors expressed *gag* from the HIV-1 strain CAM-1, *pol* from the HIV-1 strain IIIB and *nef* from the HIV-1 strain JR-FL. The MRKAd5 vaccine utilized in the STEP trial did not reduce HIV infection rates and did not decrease viral loads in individuals that became infected with HIV. On the contrary, vaccinees had a significantly higher risk for acquiring HIV as compared to the placebo group. The concomitant Phambili study aimed at testing the MRKAd5 vaccine in South Africa, where HIV-1 clade C is predominant, was stopped prematurely due to the STEP trial results. However, individuals that were already vaccinated in the Phambili study continued to be followed. As in the STEP trial, vaccinees in the Phambili trial showed significantly increased acquisition of HIV infection. Increased acquisition in the vaccine groups in both trials was restricted to subgroups of individuals with high pre-existing antibody titers to Ad5 (refs. 30,32,36). A high pre-existing antibody titer to Ad5 was associated with greater susceptibility to acquiring HIV infection when they were vaccinated with the recombinant Ad5 vaccine.[32] It has been suggested that the T cell activation caused by the recombinant Ad5 vaccine in individuals with pre-existing Ad5 immunity made them more susceptible to the initial HIV infectious event.[36,82]

The fifth efficacy trial, the RV144 or Thai trial,[38] tested a prime-boost regimen based on four injections with ALVAC-HIV[83] (the vCP1521 canarypox vector expressing the HIV genes *env*, *gag*, *pro*) followed by two injections with AIDSVAX B/E (recombinant gp120 subunit vaccine). The study enrolled over 16,000 healthy adults of which over 12,000 individuals completed all vaccination visits while remaining HIV-negative through the last scheduled vaccination. The ALVAC/AIDSVAX prime-boost vaccine did not have significant protective effects against acquisition when analyzed by a per-protocol analysis or with an intent-to-treat analysis. However, the modified intent-to-treat analysis showed 31% protection against acquisition with a p value of 0.04 (refs. 38,84). A statistical interpretation analysis published by Gilbert *et al*. reported less than a 78% chance of any vaccine efficacy at all.[39] It has been suggested that binding of IgG antibodies to variable regions of HIV-1 env proteins may have contributed to protective effects against HIV infection in the vaccine recipients.[40,41,43,45–47] Statistically significant "sieving" effects have also been reported.[42] However, these sieving effects included a gp120 env amino acid that was present in the vaccine being overrepresented in vaccine recipients who became infected as compared to placebo recipients who became infected. It also included selective sieving of amino acids in genes that were not even included in the vaccine.[85] There is no rational explanation for these sieving effect observations. The vaccine did not induce bnAbs, it did not elicit CD8+ cytotoxic T cell (CTL) responses[44,86] and viremia was not reduced in individuals that became infected with HIV.[38]

HVTN 505 is the sixth efficacy trial. The goal of the vaccine approach used in this trial was to test the efficacy of a DNA prime and Ad5 vector booster immunization in high-risk male or transgender individuals. Because of the results of the STEP and Phambili trials, individuals with high pre-existing immunity to Ad5 were excluded from the study. The HVTN 505 study was prematurely stopped due to futility 24 months after initial enrollment of participants. Therefore, data analysis could only be performed on those individuals who completed the 24-month study visits, about two thirds of the intended enrollment. The vaccine induced cellular and humoral immune responses but failed at preventing HIV infection with no difference in acquisition between the vacinees and the control group. Also, the vaccine had no influence on viral loads at set point.[37]

VACCINE TRIALS IN MONKEYS

Vaccine studies in monkeys using SIV or SHIV have been used to inform and guide the development of vaccine concepts for human clinical trials.[87–90] Results from monkey studies can be used to rank

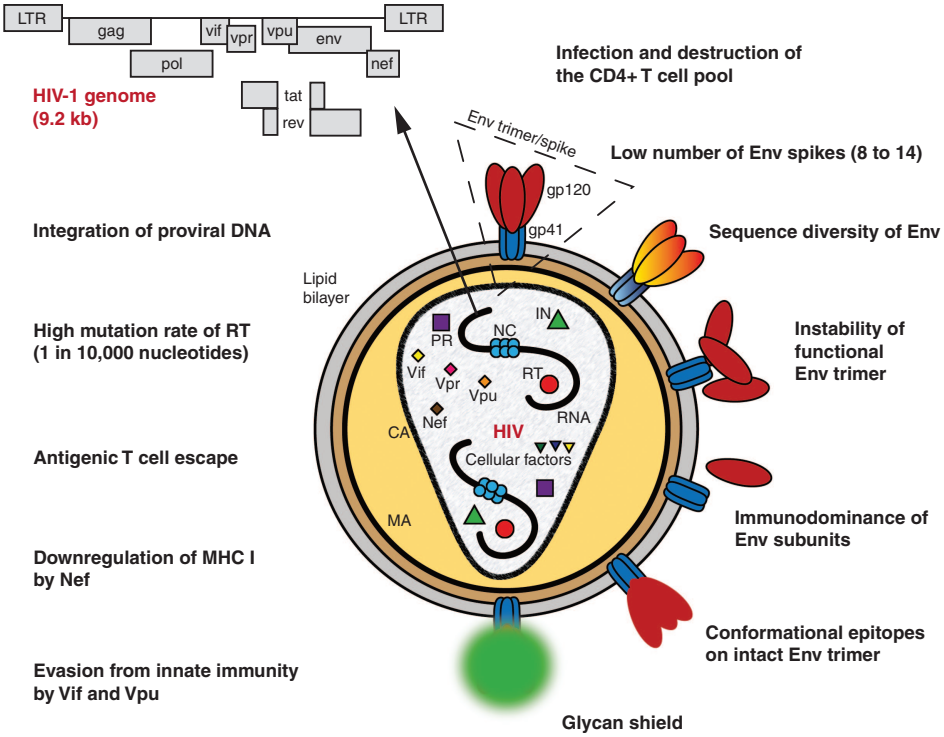

Figure 1 Difficulties associated with immune control of HIV infection. The nature of HIV and the evolution of immune evasion strategies of the virus are responsible for why a HIV vaccine has remained an elusive task. HIV preferentially infects and destroys CD4+ T cells (central mediators of the immune systems), especially in the gut-associated lymphoid tissue (GALT). The virus early establishes a reservoir in latently infected CD4+ T cells by integration of proviral DNA into the host cell genome. Recognition by cytotoxic T cells is further exacerbated by downregulation of MHC class I molecules on the surface of virus-infected cells, which is orchestrated by the viral *nef* gene. Sensing of the pathogenic intruder by the host innate immune system is counteracted by the HIV-1 genes *vif* and *vpu*. Antibody and CD8+ T cell responses are readily escaped by selection of antigenic escape variants facilitated by the high mutation rate of the virus. The error-prone reverse transcriptase causes an enormous sequence diversity of the envelope glycoproteins gp120 and gp41 (up to 35% among clades, 20% within clades, 10% in a single infected individual). An extensive glycan shield on the env trimer shields vulnerable targets on envelope (about 50% of the mass of gp120). Abbreviations: reverse transcriptase (RT); integrase (IN); protease (PR); capsid (CA); matrix (MA); nucleocapsid (NC); long terminal repeat (LTR); group-specific antigen (*gag*); the pol gene encodes RT, IN and PR; viral infectivity factor (Vif); viral protein R (Vpr); viral protein unique (Vpu); negative regulatory factor (Nef); trans-activator of transcription (*tat*); regulator of expression of virion proteins (*rev*); envelope (*env*) gene encodes the glycoprotein gp160 that is processed into gp120 and gp41.

Table 1 HIV vaccine efficacy trials in humans.

Trial	*Name of trial*	*Clinical trials identifier*	*Name of vaccine*	*Vaccine components*	*Dates*	*Population*	*Estimated enrollment*	*Efficacy*
1	VAX 004	NCT00002441	AIDSVAX B/B	gp120 proteins (clade B)	1998–2003	Adults at risk of sexually transmitted HIV-1 infection	5,400	No
2	VAX 003	NCT00006327	AIDSVAX B/E	gp120 proteins (clades B and E)	1999–2003	Intravenous drug users	2,500	No
3	STEP study (HVTN 502)	NCT00095576	MRKAd5	HIV-1 gag/pol/nef trivalent Ad5 vector vaccine	2004–2007	Adults at risk of sexually transmitted HIV-1 infection	3,000	Enhanced acquisition
4	Phambili study (HVTN 503)	NCT00413725	MRKAd5	HIV-1 gag/pol/nef trivalent Ad5 vector vaccine	2007–2007	Adults at risk of sexually transmitted HIV-1 infection	800 (of 3,000)	Enhanced acquisition
5	RV144 (Thai trial)	NCT00223080	ALVAC-HIV and AIDSVAX B/E	Canarypox vector (HIV-1 env/gag/pro), and gp120 proteins (clades B and E)	2003–2009	Adults at risk of sexually transmitted HIV-1 infection	16,400	*
6	HVTN 505	NCT00865566	VRC DNA/rAd5	DNA plasmid (gag/pol/nef/env), and rAd5 (gag/pol/env)	2009–2013	Men/Transgender at risk of sexually transmitted HIV-1 infection	2,500	No

*Per-protocol analysis: no significant efficacy; intent-to-treat analysis: no significant efficacy; modified intent-to-treat analysis: 31% efficacy ($P = 0.04$).

order, or select, the most promising concepts for trials in humans. The SIV strains SIVmac239 (ref. 91) and SIV251 (ref. 92), as well as the SHIV strains SHIV-SF162 (refs. 93,94) and SHIV-AD8 (refs. 95,96) have been preferentially used, but by no means exclusively.[22,88,97] The greatest protective efficacy in monkeys has been achieved by using live attenuated strains of SIV, such as those deleted of the *nef* gene.[97,98] Durable protection has been consistently demonstrated against homologous virus challenge in a variety of studies.[98–105] However, considerably less protection has been observed by live attenuated strains when the challenge virus was not closely matched in sequence.[105–111] This relatively unimpressive level of protection by live attenuated SIV against challenge by a heterologous AIDS virus strain is perhaps analogous to the inability of human infection with wild-type strains of HIV-1 to routinely protect against superinfection by different strains of HIV-1 (refs. 112–116).

The next most impressive degree of protection in monkey vaccine trials has been achieved with a recombinant, replication-competent herpesvirus derived from the simian cytomegalovirus (CMV).[117–119] Approximately 50% of vaccinated monkeys have shown a remarkable degree of virological control following stringent SIVmac239 challenge,[118] and no detectable signs of virus infection after more than 1 year from the time of infectious exposure.[119] The protective effects that were induced by the recombinant CMV vaccine have been associated with broad and unusual effector memory CD8+ T cell responses that recognize non-classical SIV epitopes including those that are restricted by class I antigen E or class II major histocompatibility complex molecules.[120,121] This type of immunogenicity has been found to be a result of the gene-deleted rhesus CMV strain 68-1 that was being used.[122,123] However, even the rhesus CMV vaccine conferred no protection against acquisition of the homologous challenge virus and 50% of the vaccinated monkeys showed no protective effects at all.

A variety of vector-based approaches are being examined in monkey testing and some have already advanced to human trials.[124–127] A replication-competent vector based on adenovirus type 26 (Ad26) has shown promise in protecting monkeys against stringent SIVmac251 infection[128,129] and a version expressing HIV-1 env protein has advanced to phase I clinical trials in humans.[130–132] Vectors based on rhesus monkey rhadinovirus, a gamma-2 herpesvirus that is closely related to human Kaposi's sarcoma-associated herpesvirus, are also being used in monkey trials.[133,134] Other promising viral vectors that have shown significant protective effects against SIV challenge in monkeys include: modified replicating vaccinia virus Tiantan,[135] modified vaccinia Ankara virus,[136,137] live recombinant vesicular stomatitis virus, and Semliki Forest virus replicon.[138]

BROADLY NEUTRALIZING ANTIBODIES AGAINST HIV

Following infection with HIV-1, the anti-HIV antibodies that appear over the first 3 to 6 months typically show very strain-specific neutralizing activity, specific for the sequence of the infecting strain of virus.[68,139–141] These strain-specific neutralizing antibodies target the most variable regions of the envelope protein, the so-called variable loops, principally V1 and V2 (refs. 139,142). Just as HIV can easily escape a single antiviral drug, HIV variants appear within months that resist neutralization by the early strain-specific neutralizing antibody response.[140,141,143,144] While the B cell repertoire evolves and changes in response to the changing virus, it is a race that the B cells do not win.[140] On rare occasions, however, antibodies with superior neutralization potency and breadth do emerge.[145–148] These potent broadly neutralizing antibodies (bnAbs) emerge on these rare occasions over a prolonged period of years and frequently have unusual structures that allow them to target the concealed, conserved regions of the envelope protein.[149,150]

Numerous attempts to induce bnAbs by vaccination in humans have not been successful.[151] If continually replicating HIV during the long course of infection does not routinely induce bnAbs, it is easy to imagine how difficult it will be to design immunogens to do so. The long-lasting antibody-virus chase continuum that results in these rare and potent bnAbs is consequently associated with unusual characteristics, including: a highly-evolved, high degree of somatic hypermutation (SHM) that can be accompanied by insertions and deletions; very long complementarity determining regions 3 (CDR3s); unusual structures.[149,152–156] Despite progress in areas such as reverse or structure-assisted vaccinology, it will remain an enormous challenge to those interested in antigen design and vaccine delivery to overcome these obstacles for developing a truly effective HIV vaccine.[72,157–170]

Given the difficulties in eliciting antibodies with potent neutralizing activity against a broad range of HIV-1 isolates, considerable interest has emerged in the concept of directly delivering the unusual monoclonal antibodies (mAbs) with the desired properties. More than a dozen distinct, potent bnAbs have now been isolated and characterized from infected humans (Figure 2). They can be roughly categorized into at least five groups: CD4 binding site; mannose patch; the membrane-proximal external region on gp41; Apex; the gp120-gp41 interface. The reader is referred to a number of outstanding reviews on the properties of these mAbs.[78,144,149,150,167,171,172] We may not know how to elicit such antibodies, but we already have this impressive array of potent bnAbs, they are human in origin, and they can be delivered for prevention or therapeutic purposes.

The discovery of bnAbs can historically be divided into two phases. In the early 1990s, hybridoma and phage display methods were used to isolate the first bnAbs by adsorbing sera of HIV-infected patients with monomeric gp120 and gp41 antigens. These "first-generation" bnAbs could effectively neutralize clade B viruses at a half-maximal inhibitory concentration range (IC50) of 1 to 10 µg/ml as assessed by *in vitro* assays, but they were less or not effective against other global HIV isolates.[144] Among the first-generation bnAbs were b12, 4E10, 2F5, and 2G12 (refs. 173–182). In the year 2009, the discovery of a second wave of bnAbs began following the development of improved mAb isolation techniques and the screening of larger cohorts of HIV-infected individuals. Selective B cell sorting and B cell capture methods have facilitated the isolation of a spectacular array of potent bnAbs.[144,149,167,171] These "second-generation" bnAbs are broader and two to three orders of magnitude more potent than the earlier generation of neutralizing antibodies.[144,149] Among the new bnAbs are PG9 and PG16 (ref. 183), VRC01 (ref. 184), 3BNC117 (refs. 185,186), PGT121 and PGT128 (refs. 187,188), 10-1074 (ref. 189), 10E8 (refs. 190,191), 35O22 (ref. 192), PGDM1400 (ref. 193), and VRC34.01 (ref. 194).

Passive transfer of first-generation bnAbs has conferred protection against SHIV infection in monkeys; protective effects seen in those experiments could be attributed to both the neutralization activity and the Fc-mediated effector functions of the utilized mAbs.[195–203] Consistent with these second-generation bnAbs exhibiting much higher potency in cell culture, they also showed a higher efficacy *in vivo* as compared to the first-generation bnAbs.[204] Either 3BNC117 or 10–1074, which were given to healthy macaques, were capable of completely blocking SHIV acquisition following a single intrarectal challenge with three half-maximal animal infectious doses (AID50), as long as the infused mAb dose was above 5 mg/kg.[58] A prevention study in monkeys that was published by the

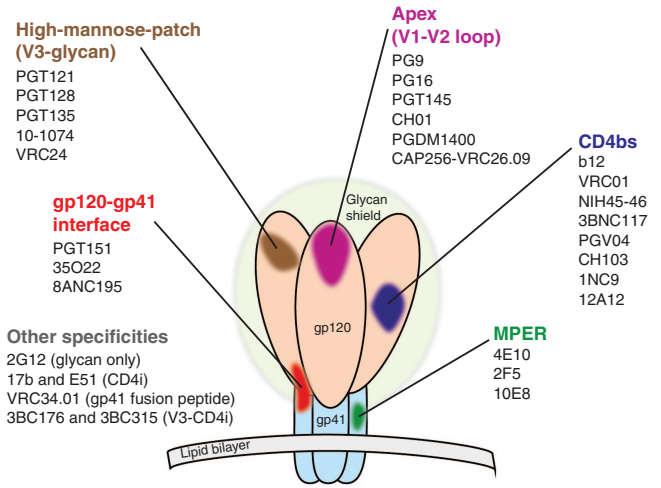

Figure 2 Broadly neutralizing antibodies (bnAbs) to HIV-1. The HIV envelope (env) spike consists of three gp120-gp41 heterodimers that are noncovalently linked to each other. The glycoprotein gp120 harbors the CD4 receptor binding site (CD4bs) and the coreceptor binding site, but the co-receptor binding region is only fully exposed upon binding of gp120 to CD4. The glycoprotein gp41 anchors the env spike into the virus membrane and harbors the fusion machinery that facilitates entry into the target cell. The env trimer spike is considered to be unstable, and decayed or nonfunctional structures appear to be a target for binding/non-neutralizing antibodies. Neutralizing antibodies, especially bnAbs strongly bind the native or functional env trimer spike. Several vulnerable bnAb target sites have been identified and a number of bnAbs bind to at least 5 well-characterized locations on the env trimer. The high-mannose-patch is located on the outer region of gp120, centered on glycans at Asn^{332} (N332). bnAbs to this site bind and penetrate the glycan shield and interact with amino acids in the variable loop 3 (V3) of gp120. Apex antibodies bind to lysine-rich regions on the V2 loop, surrounded by glycans at Asn^{160} (N160); antibodies to this site require long penetrating heavy chain structures. CD4bs antibodies have structural features that allow binding to the env trimer similar to that of the outer domain of CD4. The CD4bs is protected by the glycan shield and variable loops. MPER-specific antibodies usually have a hydrophobic character due to their binding target that is in close proximity to the lipid bilayer, which is partly recognized by this antibody class. These antibodies are usually self-reactive. Antibodies to the gp120-gp41 interface interact with both glycoproteins and appear to be trimer-specific. Abbreviations: CD4-induced (CD4i), membrane-proximal external region (MPER).

same group estimated that a 1:100 neutralization titer in plasma, which was generated by passively transferred bnAbs, was sufficient to provide protection in 50% of SHIV-exposed animals.[205]

The mAb PGT121 was tested for its protective efficacy against vaginal SHIV infection. All 10 monkeys that received a PGT121 dose of ≥1 mg/kg showed sterilizing immunity against a single high-dose SHIV-SF162P3 challenge with 300 half-maximal tissue culture infectious doses (TCID50), and three of five monkeys were even protected with a mAb dose of 0.2 mg/kg.[206] A modified version of the bnAb VRC01 with mutations in the IgG Fc portion, termed VRC01-LS, exhibited a threefold longer half-life in serum and increased translocation to mucosal tissues than unmodified VRC01 (ref. 207). The improved biochemical properties together with the overall potency of VRC01-LS provided superior protection against single high-dose rectal challenge with the strain SHIV-BaLP4 (refs. 207,208). Another study utilized the bnAbs VRC01, VRC01-LS, 3BNC117, and 10–1074 to evaluate protective efficacy against SHIV-AD8 acquisition. It was shown that monkeys that received a single mAb by passive transfer required up to 23 weekly low-dose virus challenges to become infected as compared to the control group that became infected after only a median of three challenges.[209]

Experiments in humanized mice and in monkeys have also demonstrated therapeutic potential of second-generation bnAbs. Infant rhesus macaques were infected with SHIV-SF162P3 by the oral mucosal route and treated as early as 1 day after virus infection with a mix of the bnAbs PGT121 and VRC07. Unlike the untreated animals, the mAb-treated animals were free of virus in plasma and tissue by day 14 and remained free of virus even 6 months after the infectious exposure.[56] A separate study employed monkeys that had been chronically infected with SHIV-SF162P3 for 9 months and subsequently infused with mAb cocktails containing b12, 3BNC117, and PGT121 (ref. 57). In the vast majority of animals, plasma viral loads were reduced within 7 days to undetectable levels until a median of 56 days; viremia rebounded when mAb levels decreased to sub-threshold levels. A reduction of cell-associated virus was also noted. In a parallel study, mAb treatment was employed in monkeys 3 months after SHIV-AD8 infection.[58] Monotherapy with the bnAbs 3BNC117 or 10–1074 resulted in a rapid decline in viral loads reaching undetectable levels by 4 to 7 days, followed by virus rebound that identified escape mutants to the single mAbs. A single treatment using both mAbs together suppressed viremia for 3 to 5 weeks, and readministration of the mAb combination allowed repeated transient suppression of viremia.

Monotherapy with PG16, NIH45-46^{G54W}, PGT128 or 10–1074 resulted in transiently reduced viral loads in humanized mice infected with the strain HIV-1_{YU2} (ref. 53). Virus rebound was associated with distinct escape mutations in the envelope gene. However, a single injection of a combination of bnAbs was capable of controlling HIV infection and suppressing viremia to levels below the limit of detection.[53] Viral escape from one mAb is somewhat predictable, as the selective immune pressure is not sufficient to inhibit viral replication long-term. Based on *in vitro* neutralization assays and mathematical prediction models, it has been reported that a combination of three to four potent bnAbs is likely to provide complete or near complete protection against HIV replication.[210,211] Another study that utilized a similarly combined passive transfer regimen involving the mAbs PG16, 10–1074, and 3BNC117 confirmed suppressive effects on HIV in humanized mice, which included lowering of free virus in serum, delayed viral rebound after cessation of antiretroviral therapy (ART) and reduction of cell-associated HIV-1 DNA.[54]

Although ART and multiple bnAbs are able to suppress viremia in infected mice, there are still latent reservoirs of HIV-infected cells that are refractory to those treatments. An approach, called "shock and kill", that combines ART and inducers of viral transcription has so far failed to eradicate the latent HIV reservoir.[212] However, a study in mice showed that a trimix of bnAbs in combination with three inducers was capable of decreasing the HIV reservoir as measured by viral rebound. Interestingly, the data also revealed that suppression of HIV by the passively transferred Abs was dependent on interaction of the IgG Fc with Fc receptors of immune cells suggesting the importance of IgG effector functions.[55] Other studies confirmed that Fc receptor-mediated effector functions of bnAbs play a substantial role in inhibiting HIV or SHIV infection.[200,213–215] In this context, the antiviral activity of the IgG Fc is directed against both free virus and virus-infected cells. Therefore, the potency or antiviral capacity of an anti-HIV Ab is not only defined by the affinity function of its Fab but also by the effector mechanisms that are mediated by its Fc.[72,78,144,216,217]

Some first-generation and second-generation bnAbs have also progressed to human clinical trials. When used in HIV-positive individuals as therapy, first-generation bnAbs were well tolerated

and appeared to be safe at doses up to 14 g of mAb over a 4-week period.[218–220] Passive administration of 2F5 and 2G12 resulted in a transient reduction of viral loads in five of seven patients; the median decrease of RNA copies/ml in plasma was about 1 log during the treatment phase (day 0–28) while the maximum decrease was 1.5 log.[221] In a subsequent study, the effect of three bnAbs was tested in a human clinical trial. The goal of the experiment was to examine antibody-mediated suppression of HIV-1 rebound after cessation of ART.[222] Sequential infusions of the mAbs 2G12, 4E10, and 2F5 to HIV-infected individuals undergoing interruption of ART showed a delay in viral rebound. Passively administered antibodies showed a substantial inhibitory effect in two of eight chronically infected and in all six acutely HIV patients as compared to a control group, and viral rebound was significantly delayed in acutely infected subjects that received mAb therapy versus those that did not receive mAb therapy. The authors also noted that the bnAb 2G12 had the strongest antiviral effect of all three mAbs used, and that the loss of viremia control in 12 of the 14 immunized patients was associated with viral escape from that mAb. No escape mutants were noted for the other two mAbs, 4E10 and 2F5. Another group conducted a similar passive transfer experiment using the same three bnAbs and confirmed the previously obtained results.[223]

Phase 1 trials have now evaluated safety, pharmacokinetics and functionality of second-generation bnAbs in people as well. The bnAbs VRC01 and 3BNC117 have been among the first of these to prove their potency in humans; results with these mAbs were just published in the year 2015. Twenty-eight healthy volunteers were given intravenous infusions of the mAb VRC01 (ref. 224). VRC01 appeared to be safe and well tolerated; also, no serious adverse events and no dose-related toxicities were noted following the mAb infusions. The mean concentrations over a 28-day period were 35 µg/ml (at 20 mg/kg) and 57 µg/ml (at 40 mg/kg); readministration on day 28 increased the mean concentration in serum to 56–89 µg/ml; the half-life of VRC01 was 15 days. Furthermore, no anti-VRC01 antibody responses were detected in any volunteer at any time. In another human trial, VRC01 was given to HIV-infected individuals. ART-treated and ART-untreated HIV patients received infusions of the VRC01 mAb at a dosage of 1, 5, 20, or 40 mg/kg.[59] Two mAb infusions, conducted on day 0 and 28, did not reduce the amount of cell-associated viral DNA (also referred to as reservoir) in the ART-treated HIV patients with undetectable viral loads in plasma. However, a single infusion of VRC01 decreased the plasma viral load by 1.1–1.8 $\log_{10}$ in six of the eight ART-untreated viremic HIV patients. Reduction of viremia was transient and viral loads returned to baseline levels within 56 days after mAb infusion due to waning mAb levels and selection for less sensitive viruses.

Another human trial that employed passive transfer was published in the same year. The bnAb 3BNC117 was tested in 12 healthy and 17 HIV-infected individuals.[60] A single infusion of the mAb appeared to be safe and well tolerated at all doses tested (1, 3, 10, 30 mg/kg); also, no serious adverse events were noted. The half-life of 3BNC117 was 17 days in healthy volunteers and 9 days in HIV-infected patients. HIV-infected individuals that received lower doses of 3BNC117 showed only small and transient reductions in viral loads followed by a rapid return to baseline levels. However, a single infusion of the mAb at higher doses (10 and 30 mg/kg) reduced the viremia up to 2.5 $\log_{10}$ in 10 out of 11 subjects, and viral loads remained significantly reduced for 28 days. Emergence of resistant viral strains was variable among the 3BNC117 recipients. Development of increased neutralization resistance was observed in some patients that exhibited escape mutations in the CD4bs and amino acid insertions in the V5 loop of HIV env.

Further experiments were conducted to explore the antiviral capacity of the bnAb 3BNC117 (ref. 225). Suppression of viral load was attributed to clearance of free virus and reduction of virus spread by clearance of virus-infected cells; clearance of virus-infected cells was dependent on Fc-mediated effector functions of 3BNC117. Another study examined the effects of 3BNC117 monotherapy on the host's antibody responses.[226] Autologous IgG samples from day 0 and week 24 postinfusion were tested for their capacity to neutralize a panel of HIV-1 pseudoviruses and autologous viruses from day 0 and week 4 postinfusion. It was shown that autologous week 24 IgG, by which time 3BNC117 had already decayed to below detection, had an increased neutralizing activity against weeks 0 and 4 autologous viruses as compared to the neutralizing activity of autologous day 0 IgG. Therefore, patients that received a passive immunization against HIV appeared to develop stronger host antibody responses to their own HIV infection. A separate trial investigated the effects of 3BNC117 on HIV after ART interruption. The results showed that repeated mAb administrations significantly delayed virus rebound as compared to nontreated individuals; but it also revealed that virus rebounded after antibody levels waned, and that use of 3BNC117 alone led to neutralization-resistant escape mutants.[227]

Although it has been shown that sera from HIV-infected individuals can enhance HIV infection *in vitro*, there has been no clear evidence that passively transferred antibodies pose a risk to enhancement of HIV infection *in vivo*.[228–230] Nonetheless, antibody-dependent enhancement could theoretically represent a problem to passive immunization strategies against HIV. Despite the promise of utilizing bnAbs to prevent or treat HIV infection, reasonable risk assessments will need to be performed for each individual anti-HIV mAb to exclude the chance of increased virus acquisition or increased virus replication following passive transfer to humans.[229]

AAV-MEDIATED DELIVERY OF ANTIBODIES AND ANTIBODY-LIKE MOLECULES

With the availability of more than a dozen potent bnAbs, and given developments in antibody engineering that have enhanced biochemical and antiviral properties,[231–247] it is easy to imagine the potential for the effectiveness of such anti-HIV mAbs in both prevention and therapeutic scenarios. In prevention scenarios, delivery of potent bnAbs could overcome the difficult barriers to trying to induce such antibodies, with the goal of creating a long-term sterilizing barrier to infection. In therapeutic scenarios, the goals would be to greatly reduce viral replication and plasma viral loads, to eliminate the need for continuing antiviral drug therapy, and it would also hope to reduce viral reservoirs over time toward a real cure.

One issue that will need to be addressed, particularly for therapeutic scenarios, is whether some particular combinations of mAbs provide remarkably synergistic levels of protective effects. Do some combinations of potent bnAbs result in a much greater degree of virus neutralizing activity than either alone?[211] Do some combinations of potent bnAbs make it much more difficult or impossible for the virus to escape the activity of the combination? Does escape from some combinations of potent bnAbs result in virus that is so poorly fit for replication that it can be easily controlled by the host? Does escape from some combinations of potent bnAbs result in virus that is so easily neutralized that it can be well controlled by the host immune responses? These questions can be readily addressed by cell culture and monkey studies.

Maintenance of effective concentrations of mAbs over prolonged periods by passive administration would require repeated, regular infusions over a prolonged period. This does not seem practically

possible on a large scale for a variety of reasons. First, it would be prohibitively expensive to use on a large scale just for the antibody production, purification and quality control. Second, long-term adherence is certainly likely to be a problem, particularly in many regions of the developing world. Recombinant adeno-associated virus (rAAV) is ideally suited to achieve the goal of long-term delivery (Figure 3). AAV-based gene delivery is considered to be a safe and effective technology.[248–257] Numerous studies in monkeys[258–261] and people[262–275] have shown the successful and safe application of rAAV vectors for the treatment of various genetic diseases. The positive results of clinical trials for lipoprotein lipase deficiency have led to the first gene therapy product to achieve regulatory approval by a governmental health institute.[276–279]

The only product that is made by rAAV derives from the transgene that was cloned into the vector.[254,280–284] Genetically engineered AAV genomes persist in the cell in episomal form and will produce your protein of choice for the lifetime of the cell.[285,286] AAV is capable of transducing quiescent cells such as those from skeletal muscle (Figure 4); as long as the transgene product is viewed as self by the host immune system, rAAV-delivered proteins can be secreted for decades from such long-lived cells.[48,49,251,261,286,287] Several groups have demonstrated the protective efficacy of AAV-delivered antibodies and antibody-like molecules against AIDS virus infection in monkeys and humanized mice.[48–50,52,288–291]

A pioneering study conducted in rhesus macaques employed AAV-delivered single-chain fragment variable (scFv) immunoadhesins (antibody-like molecules) to protect against SIV infection.[48] The genetic material encoding the scFv immunoadhesins 4L6 and 5L7 used in that experiment was small enough to be accommodated by self-complementary AAV (scAAV) vector, a recombinant AAV variant that encapsidates double-stranded DNA.[292] The scAAV vector was chosen due to reports of its enhanced transduction capability and performance at achieving higher rates of transgene expression. However, scAAV is limited at packaging longer sequences such as the genetic information of both heavy and light chain sequences of a full-length immunoglobulin G (IgG).[293–295] Conventional single-stranded AAV (ssAAV) vector was used to deliver a rhesus CD4 - rhesus IgG fusion construct, termed N4. All three vectors (4L6, 5L7, and N4) had an AAV1 capsid. Following intramuscular injection of the rAAVs, immunoadhesin concentrations in serum reached up to 190 µg/ml by 4 weeks, and levels of immunoadhesins were maintained in some of the scAAV recipients above 200 µg/ml through 12 months. The nine AAV-immunized monkeys and two groups of control monkeys were challenged with a high dose of the strain SIVmac316 at 4 weeks following the AAV gene transfer. While all six control monkeys became infected by the SIV challenge, six of the nine AAV-immunized monkeys that maintained reasonable levels of immunoadhesins showed sterile protection against SIV exposure.

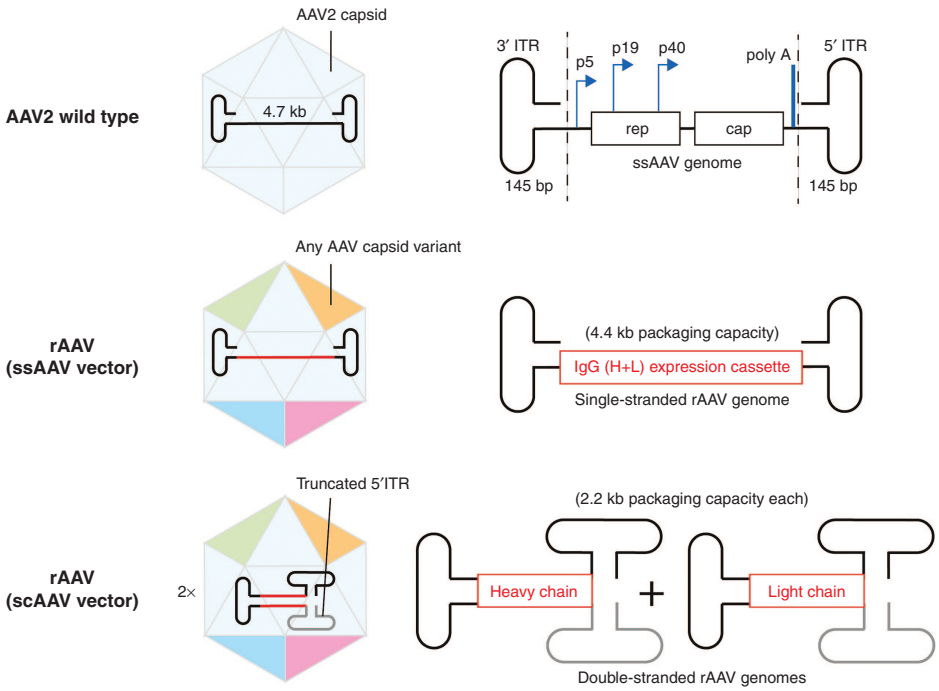

Figure 3 Recombinant adeno-associated virus (rAAV) vectors for the delivery of monoclonal antibodies (mAbs). Wild-type adeno-associated virus (AAV) is a 25 nm small nonenveloped virus that packages a single-stranded DNA genome. The most prominent AAV serotype, AAV2, has a genome size of 4.7 kb and harbors two viral genes (*rep* and *cap*) that are flanked by two 145 bp inverted terminal repeats (ITRs). Four Rep proteins (Rep78, Rep68, Rep52, and Rep40) are produced from transcripts using the p5 and p19 promoters, and these proteins are important for viral replication and regulation of AAV gene expression. The virus does not encode a polymerase enzyme and relies on cellular enzymatic activities. Furthermore, AAV relies on the presence of helper viruses such as herpesvirus or adenovirus in order to undergo productive infection (replication, gene expression, and virion production). The *cap* gene encodes three structural capsid proteins (VP1, VP2, and VP3) from two transcripts using the p40 promoter. For generating recombinant AAV (rAAV), the entire wild-type AAV genome is replaced by a unique transgene cassette (such as for a mAb) flanked by the AAV ITRs, which are the only wild-type sequences remaining. Production of rAAV virions is achieved by triple transfection using the rAAV vector plasmid and two helper plasmids in *trans*, followed by CsCl purification of the replication-deficient rAAV particles. rAAV particles can be encapsidated by any of the 12 AAV serotypes and more than 100 variants that are available. The conventional single-stranded AAV (ssAAV) vector packages single-stranded DNA. The modified self-complementary AAV (scAAV) encapsidates double-stranded DNA but has only half the packaging capacity of ssAAV. scAAV vectors are produced by modification of the 5′ITR (removal of the terminal resolution site and D sequence). The size limit of the scAAV vector system requires the heavy and light chain sequences of IgG to be placed on separate rAAV vectors.

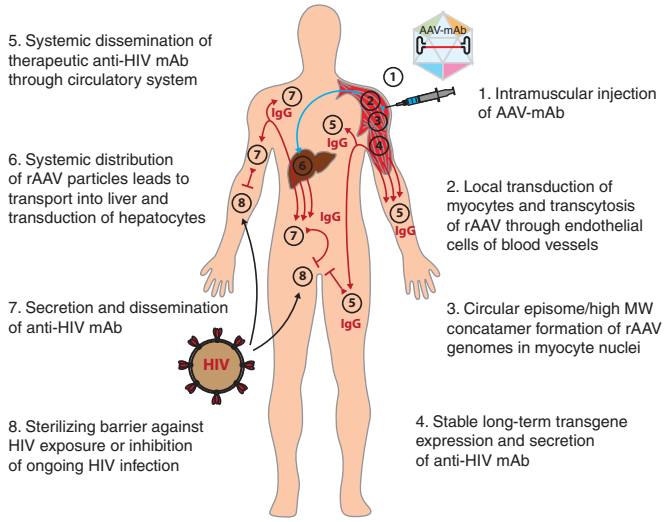

Figure 4 AAV-mediated gene transfer of anti-HIV monoclonal antibodies (mAbs). A rAAV encoding an anti-HIV mAb is injected into muscle such as the deltoid muscle. Following intramuscular inoculation, rAAV binds to a serotype-specific cellular receptor on myocytes and becomes endocytosed. Virus particles are transported to the nucleus, into which the rAAV genome is released. The single-stranded DNA (in case of ssAAV) is then converted into transcriptionally active double-stranded DNA. Double-stranded rAAV genomes appear to be stabilized by ITR-to-ITR interactions and enzymatic modifications, leading to high molecular weight (MW) rAAV genome polymers that persist in episomal form for the lifetime of the cell. Adult human muscle cells have a lifespan of more than 10 years. The extrachromosomal rAAV DNA forms maintain gene expression and produce the therapeutic mAb, which undergoes the secretory pathway and is released into the circulatory system. Depending on which AAV serotype or variant is used, the injected rAAV virus can also transcytose through multiple cell layers, leading to access to blood vessels. This will transport a proportion of intramuscularly injected rAAV particles to the liver, where cell entry and gene expression will take place. Secretion of an anti-HIV mAb from muscle and liver will potentially create a prophylactic barrier against HIV infection or fight an ongoing HIV infection in a therapeutic setting. AAV, adeno-associated virus; ITR, inverted terminal repeats.

However, the three other AAV-immunized monkeys developed immunoadhesin-specific antibody responses, which incapacitated protective efficacy and led to SIV infection.

Our group set out to build on that study by attempting to deliver authentic IgG versions of the rhesus-derived 4L6 and 5L7 (ref. 296). We wished to address the question whether the delivery of authentic full-length versions of these antibody-like molecules could obviate the anti-antibody response to them. Since both heavy and light chain sequences could not be accommodated by one scAAV, we placed them on two separate scAAV vectors. Additionally, we placed both heavy and light sequences onto one ssAAV vector by utilizing F2A peptide technology as previously described.[297,298] Two scAAV vectors or a single ssAAV vector, encapsidated by AAV1, were injected intramuscularly into 12 rhesus macaques and levels of AAV-delivered Abs were measured over time.[49] The concentration of the SIV-specific antibodies in serum ranged from 1 to 270 µg/ml through 44 weeks, regardless which AAV vector delivery system was being used. However, the conversion to authentic IgG sequences did not prevent the emergence of anti-antibody responses and this emergence limited the concentration of the SIV-specific antibodies that could be achieved. Nonetheless, we progressed with a challenge phase and conducted a repeated low dose challenge regimen using the neutralization-resistant strain SIVmac239. Although 4L6 and 5L7 IgGs showed no neutralizing activity *in vitro*, they exerted antiviral effects against highly pathogenic SIVmac239 challenges *in vivo* as assessed by the significant delay and reduction of viremia in plasma.[49]

Another study conducted in monkeys utilized AAV-antibody gene transfer to protect against SHIV infection.[51] Rhesus macaques were injected intramuscularly with a ssAAV8 vector expressing the potent bnAb VRC07. In four of four test animals, the serum concentration of the AAV-delivered antibody reached 8 µg/ml by week 4 and plummeted to undetectable levels by week 9. This was apparently due to a vigorous anti-VRC07 antibody response despite extensive attempts to make the VRC07 mAb as "rhesusized" as possible. A second group of monkeys then received the immunosuppressive agent cyclosporine A (CsA) prior to the AAV-antibody gene transfer. Although levels of delivered VRC07 reached serum concentrations as high as 66 µg/ml by week 3, anti-VRC07 antibody responses lowered the AAV-delivered antibody during and especially after immunosuppression. Following challenge with the strain SHIV-BaLP4, significantly more control monkeys became infected as compared to the AAV-antibody group.

One of the most potent and broad molecules capable of inhibiting AIDS virus entry is the antibody-like construct eCD4-Ig.[50] This molecule is composed of the outer two domains of CD4 (entry receptor of the AIDS virus), the Fc portion of IgG and a CCR5 (entry coreceptor of the AIDS virus) mimetic peptide that is derived from the HIV-specific antibody E51 (refs. 299–302). While some significant number of HIV-1 isolates are resistant to neutralization by potent bnAbs, eCD4-Ig has neutralized 100% of the tested neutralization-resistant strains. Furthermore, eCD4-Ig has been shown to potently neutralize HIV type 2 and the neutralization-resistant strain SIVmac239. Following AAV1 gene transfer to monkeys, levels of the antibody-like molecule eCD4-Ig ranged between 17 and 77 µg/ml by week 30. The four AAV-immunized macaques and four control macaques were then repeatedly challenged with progressively increasing doses of SHIV-AD8. Complete protection was demonstrated in the AAV-immunized animals, while all control animals became infected following the last challenge that utilized 4 AID50. Interestingly, anti-eCD4-Ig host antibody responses were low or absent, while AAV-transferred potent bnAbs used in that study elicited moderate to strong anti-antibody responses.[50]

AAV-antibody gene transfer has also been used in humanized mice experiments. Notably, AAV-delivered bnAbs b12, VRC01 and 10–1074 have demonstrated protective effects against HIV acquisition and durable control of HIV in a therapeutic setting.[52,54,291] Although mouse experiments can demonstrate whether potent bnAbs have the ability to block or inhibit HIV infection *in vivo*, the humanized mouse model has certain limitations and may fall short when evaluating HIV pathogenesis, as well as safety and immunogenicity of AAV-delivered antibodies. Since virus challenge experiments are performed in immunocompromised mice that have been engrafted with human cells, it is difficult to translate results to immunocompetent monkeys or humans.[88]

One trial is currently ongoing to evaluate safety, deliverability, and potential efficacy of rAAV-delivered potent bnAbs: PG9 in uninfected human volunteers in England.[127]

OBSTACLES FOR EFFICIENT AAV-ANTIBODY DELIVERY

Several features of the rAAV vector delivery system may serve to limit the effectiveness with which the desired protein can be expressed. As with any virus, AAV can be recognized as foreign by the host immune system.[303–309] While rAAV vector does not directly express any wild-type AAV proteins, rAAV on its own may trigger

innate immune responses.[307] Furthermore, pre-existing cellular[308] and humoral[309] immunity to wild-type AAV may significantly limit the ability of the rAAV to "take" in the host.[310]

There are 12 AAV serotypes and more than 100 variants (serovars) as specified by phylogenetic analyses.[252,311–313] A number of studies have reported that individual AAVs can be sensed by pattern recognition receptors (PRRs), which can lead to upregulation of host defense genes and the production of proinflammatory cytokines and chemokines. This in turn will activate cells of the innate immune system and may amplify the inflammatory signal by initiating adaptive immune responses. Toll-like-receptors (TLR) such as TLR-2 and TLR-9 have been shown to be involved in innate immune responses to AAV by recognizing the AAV capsid and the AAV genome, respectively.[314–318]

The prevalence of anti-AAV capsid IgG in the human population varies by AAV serotype; *e.g.*, up to 72% of people are sero-positive for AAV2, 67% for AAV1 and 38% for AAV8. Also, antibodies against one serotype may cross-react against another serotype depending on how similar their capsid sequences are.[319–321] Neutralizing antibodies in serum at titers of more than 1:5 may already be sufficient to capture intravenously injected virus particles, and by so doing severely reduce transduction by rAAV.[262,322–324] Furthermore, several groups have explored the possibility of AAV re-administration to muscle with moderate or no success when the exact same serotype was used[325–327]; only in the event of immunosuppressive or immunomodulatory intervention was it possible to achieve effective uptake of the same AAV serotype.[328–330] Similarly, presence and activation of AAV capsid-specific memory CD8+ T cells can eliminate cells that have taken up rAAV particles.[331] Human trials that have employed rAAV to provide functional protein to individuals with hereditary disorder have reported anti-capsid responses to AAV2, AAV8 and AAV1 (refs. 263,264,269). AAV gene transfer may also elicit immune responses against the rAAV-delivered transgene product if the host has never seen that specific protein; in that context, the magnitude of the response is dependent on the degree the endogenous gene is different from the delivered gene, in particular whether the host protein may be truncated or missing entirely.[332–336] Human trials have reported transgene-specific cellular responses against rAAV-delivered α1-antitrypsin (AAT), mini-dystrophin protein and coagulation factor IX (F9).[263,269,332,337–339] Furthermore, the magnitude and frequency of immune responses to rAAV vector and delivered transgene product is influenced by several other factors including the AAV serotype or variant that is being used, rAAV tropism for antigen-presenting cells (APCs), the rAAV dose and the route of rAAV administration.[332,340–350]

When considering use of rAAV for delivery of mAbs, the first inclination is to assume that human antibodies are natural protein products of humans and should therefore not be viewed as foreign. However, things may not be that simple. The human B cell repertoire can create an enormous number of different antibodies with enormous sequence variation.[165,351,352] A particular antibody being made by one individual will not likely ever have been seen by another individual and will likely be less tolerized by the other individual. Furthermore, any particular antibody being made by an individual must have been accepted by a complex checkpoint system during B cell development within that host.[353–356] These considerations are further exaggerated by the highly evolved, highly mutated nature of the potent bnAbs one wants to deliver for the prevention or treatment of HIV infection.[150] bnAbs have undergone extensive SHM in their variable domains, which allows them to attain enhanced antiviral potency and breadth, but this may also be associated with some self-reactivity and with immunogenicity.[154,357–360] CDR-sequence containing regions of variable domains of IgGs (idiotypic variation) may contain CD4+ T cell epitopes that induce unwanted immune responses in the mAb recipient.[361,362] Other properties of mAbs may also contribute to their immunogenicity in the recipient host: allotypic variation, misfolding, aggregation and differences in glycosylation.[363,364] Immune responses following passive transfer to humans have been reported for a number of therapeutic mAb.[365–368] Although species-specific antibodies have shown to have less immunogenic potential, immune responses to mAbs in humans have occurred independently of the nature of the transferred mAb: murine versus humanized versus fully human.[365,369–371]

There have been five monkey trials to date where rAAV has been used to deliver antibodies or antibody-like molecules against HIV or SIV. The pioneering study by Johnson *et al*.[48] utilized rAAV1 to delivery the antibody-like molecules (immunoadhesins) 4L6, 5L7, and N4 as prophylaxis against SIV challenge. In contrast to the heavy and light chain coding sequences of a full-length mAb, the coding sequence of an immunoadhesin is small enough to be accommodated by scAAV; this vector type was being used for 4L6 and 5L7. Three of nine rhesus monkeys developed anti-immunoadhesin responses, and these three monkeys were not protected from SIV infection. Although 4L6 and 5L7 are composed of fully rhesus-derived sequences, humoral responses targeted these sequences. The authors found that reactivity was confined to the variable domains of these two immunoadhesins. Humoral responses were also measured against the rhesus CD4 moiety of N4, albeit modest. It is worth noting that 4L6 and 5L7 are extremely hypermutated and bear very long CDR3 sequences. Also, sequences of heavy and light chains were obtained by phage display, which might not resemble a natural pairing of these chains. The artificial fusion of variable light (VL) and variable heavy (VH) domains, as well as CD4 with the IgG Fc could have potentially created conformational epitopes that could be immunogenic.[372] Our group converted those immunoadhesin sequences to authentic IgG molecules to potentially avoid any unnatural structures.[296] However, Fuchs *et al.*[49] and Martinez-Navio *et al*.[359] found that full-length IgG versions of 4L6 and 5L7 did not prevent anti-antibody responses. Six out of six monkeys that received 4L6 IgG1 and three out of six that received 5L7 IgG1 generated anti-antibody responses. Both heavy and light chain variable regions were targeted including measured reactivity to the heavy chain CDR3 (ref. 359).

Our group has also delivered rhesusized versions of anti-HIV bnAbs (1NC9, 8ANC195, 3BNC117, 10–1074, and 10E8) to monkeys; anti-antibody responses were readily detected against all AAV-delivered antibodies in all eight animals.[359] The levels of delivered mAbs were driven to below detection in all animals for all antibodies for which specific detection methods were available. Immunogenicity of the tested anti-HIV bnAbs correlated significantly with the degree of sequence divergence from germline.[359] In another study, Saunders *et al*.[51] delivered the HIV-specific bnAb VRC07 using AAV8. Four of four monkeys elicited anti-antibody responses to the mAb, and these unwanted anti-antibody responses resulted in a loss of transgene product in all animals by 9 weeks following rAAV administration.[51] It is worth noting that the vigorous anti-antibody responses were mounted against VRC07, despite extensive efforts to "rhesusize" the mAb as much as possible. The bnAb VRC07 was created by pairing the light chain of the bnAb VRC01 with a heavy chain isolated from a B cell clone of the VRC01 lineage.[373] This unnatural pairing of heavy and light chains, along with the 14% SHM rate of VRC01 (ref. 167) (full-length antibody sequence as compared to full-length germline

sequence) and the further mutated VRC07 heavy chain sequence, may have contributed to the immunogenicity of VRC07. In a second group of animals, use of CsA did not prevent anti-VRC07 antibody responses, but humoral responses were blunted in three of six monkeys by that immunosuppressive intervention, and those three monkeys maintained measurable mAb levels through 16 weeks.[51]

Gardner *et al*.[50] delivered the broad and potent anti-HIV entry inhibitor eCD4-Ig by AAV1 to monkeys. While two of four monkeys had a weak anti-eCD4-Ig response, the other two showed no detectable anti-inhibitor reactivity. Comparably modest anti-inhibitor responses have also been observed with N4 (ref. 48). Since rhesus CD4 and rhesus IgG Fc are self proteins to rhesus monkeys, no considerable humoral responses were elicited.[50] Furthermore, no reactivity was raised against the CCR5 mimetic peptide, a CDR3-derived peptide[299,301] that was artificially fused to the IgG Fc.[50] Apparently, the amino acid sequence and the arrangement of the CCR5 mimetic peptide have not presented a major immunogenic stimulus in monkeys. The same group also tested the immunogenicity of the AAV-delivered bnAbs 3BNC117, NIH45-45, 10–1074, and PGT121 in monkeys.[50] The bnAbs elicited significantly higher anti-antibody responses as compared to eCD4-Ig. The rate of SHM among those 4 bnAbs is relatively high: 3BNC117 (36.9%), NIH45-46 (44%), 10–1074 (24.4%), and PGT121 (21.2%).[167]

The inherent nature of an anti-HIV bnAb may be sufficient to elicit immune responses in the recipient host since the recipient likely never would have generated or experienced the specific variable domains. Human mAbs used therapeutically have been shown to elicit immune responses in a substantial fraction of humans following passive transfer.[365,366,368] To our knowledge, no side-by-side comparison has been conducted that evaluates the immunogenicity of a mAb when administered passively versus by AAV gene transfer. The anti-HIV bnAb VRC01 did not appear to elicit anti-VRC01 antibody responses in humans following one or two administrations.[224] A simianized version of VRC01 elicited anti-VRC01 antibodies in two of eight macaques following four passive administrations.[208] The simianized mAb VRC07 elicited robust anti-VRC07 antibody responses in four of four monkeys when delivered by rAAV,[51] similar to the experience of Martinez-Navio *et al*. with a variety of AAV-delivered rhesus and rhesusized human mAbs.[359] Again, the anti-anti responses to the AAV-delivered mAbs were directed principally or exclusively to the variable domains, *i.e.*, they were anti-idiotypic in nature.[51,359]

A number of studies have explored ways of reducing immune responses toward a variety of AAV-delivered gene products. The use of immunosuppressive agents such as CsA has shown partial success at reducing immune responses and facilitating transgene expression in monkeys.[51] Temporary inhibition of CD4+ T cells has shown to be effective at preventing immune responses against AAV-mediated gene delivery, particularly in the context of AAV readministration in mice.[330] A single patient case report showed that combined use of intravenous immunoglobulin (IVIG), B cell ablation and a corticosteroid has allowed for successful AAV-mediated gene transfer in the absence of immune responses towards AAV capsid and the delivered transgene product.[374] Passive transfer of a large dose of mAb prior to recombinant AAV administration may circumvent the problem of "inverse dose-immunogenicity relationship".[365,375] If readministration of rAAV is desired, the second AAV inoculation could employ a different serotype than the one used in the primary inoculation. Also, the use of engineered AAV capsids may help at minimizing host immune responses; AAV capsid mutations that involve Tyr, Lys, Ser, and Thr residues have shown to improve AAV transduction, and such capsid mutations could allow efficient AAV gene transfer at a lower AAV dose while potentially reducing the sensing by the innate immune system.[343,376–378] Use of specific microRNA binding sites (miRNAbs) within the rAAV genome may prevent transgene expression in professional antigen presenting cells (APCs) and thus inhibit elicitation of immune responses.[379,380] Liver-directed AAV gene transfer may accomplish induction of tolerance toward any mAb. Expression of transgene products in liver tissue has been demonstrated to be tolerogenic by mechanisms that include but are not limited to induction of regulatory T cells (Tregs).[381–386]

SUMMARY

Wild-type AAV has never been associated with the cause of any known diseases in humans, and recombinant AAV has demonstrated its overall efficacy and safety in more than 120 clinical trials, with transient tissue inflammation as the most severe side effect.[256,387] Given the need to explore unconventional approaches against HIV, AAV-mediated delivery of potent anti-HIV bnAbs represents a promising approach for the prevention and treatment of HIV infection. Trials in monkeys have demonstrated significant efficacy of rAAV-delivered antibodies and antibody-like molecules for prevention of AIDS virus infection. Nonetheless, despite the safe and effective application that has been attributed to AAV-mediated gene transfer, immune responses to AAV-delivered antibodies remain the most significant impediment that will limit the effectiveness of this approach. This impediment needs to be better understood and overcome for the promise of the AAV-antibody approach to be effectively realized in people.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Gottlieb, MS, Schroff, R, Schanker, HM, Weisman, JD, Fan, PT, Wolf, RA *et al.* (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* **305**: 1425–1431.
2. Barré-Sinoussi, F, Chermann, JC, Rey, F, Nugeyre, MT, Chamaret, S, Gruest, J *et al.* (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**: 868–871.
3. UNAIDS (2000). REPORT on the global HIV/AIDS epidemic. http://data.unaids.org/pub/Report/2000/2000_gr_en.pdf.
4. UNAIDS (2013). Global report: UNAIDS report on the global AIDS epidemic. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf.
5. Barré-Sinoussi, F, Ross, AL and Delfraissy, JF (2013). Past, present and future: 30 years of HIV research. *Nat Rev Microbiol* **11**: 877–883.
6. Esparza, J (2013). A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine* **31**: 3502–3518.
7. Barry, SM, Mena Lora, AJ, and Novak, RM (2014). Trial, error, and breakthrough: a review of HIV vaccine development. *J AIDS Clin Res* **5**: 359.
8. Girard, MP, Osmanov, S, Assossou, OM and Kieny, MP (2011). Human immunodeficiency virus (HIV) immunopathogenesis and vaccine development: a review. *Vaccine* **29**: 6191–6218.
9. Hütter, G, Nowak, D, Mossner, M, Ganepola, S, Müssig, A, Allers, K *et al.* (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* **360**: 692–698.
10. Allers, K, Hütter, G, Hofmann, J, Loddenkemper, C, Rieger, K, Thiel, E *et al.* (2011). Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. *Blood* **117**: 2791–2799.
11. Yukl, SA, Boritz, E, Busch, M, Bentsen, C, Chun, TW, Douek, D *et al.* (2013). Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. *PLoS Pathog* **9**: e1003347.

12. Siliciano, JD and Siliciano, RF (2016). Recent developments in the effort to cure HIV infection: going beyond N = 1. *J Clin Invest* **126**: 409–414.
13. Günthard, HF, Aberg, JA, Eron, JJ, Hoy, JF, Telenti, A, Benson, CA *et al.*; International Antiviral Society-USA Panel. (2014). Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA* **312**: 410–425.
14. Nakagawa, F, May, M and Phillips, A (2013). Life expectancy living with HIV: recent estimates and future implications. *Curr Opin Infect Dis* **26**: 17–25.
15. Siliciano, JD, Kajdas, J, Finzi, D, Quinn, TC, Chadwick, K, Margolick, JB *et al.* (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med* **9**: 727–728.
16. Strain, MC, Gunthard, HF, Havlir, DV, Ignacio, CC, Smith, DM, Leigh-Brown, AJ, *et al.* (2003). Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. *Proceed Natl Acad Sci USA* **100**: 4819–4824.
17. Finzi, D, Hermankova, M, Pierson, T, Carruth, LM, Buck, C, Chaisson, RE *et al.* (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* **278**: 1295–1300.
18. Wong, JK, Hezareh, M, Günthard, HF, Havlir, DV, Ignacio, CC, Spina, CA *et al.* (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* **278**: 1291–1295.
19. Chun, TW, Stuyver, L, Mizell, SB, Ehler, LA, Mican, JA, Baseler, M, *et al.* (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Nat Acad Sci USA* **94**: 13193–13197.
20. Finzi, D, Blankson, J, Siliciano, JD, Margolick, JB, Chadwick, K, Pierson, T *et al.* (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* **5**: 512–517.
21. UNAIDS (2015). AIDS by the numbers. http://www.unaids.org/sites/default/files/media_asset/AIDS_by_the_numbers_2015_en.pdf.
22. Desrosiers, RC (2004). Prospects for an AIDS vaccine. *Nat Med* **10**: 221–223.
23. Desrosiers, RC (1999). Strategies used by human immunodeficiency virus that allow persistent viral replication. *Nat Med* **5**: 723–725.
24. Lifson, JD and Haigwood, NL (2012). Lessons in nonhuman primate models for AIDS vaccine research: from minefields to milestones. *Cold Spring Harb Perspect Med* **2**: a007310.
25. Flynn, NM, Forthal, DN, Harro, CD, Judson, FN, Mayer, KH and Para, MF; rgp120 HIV Vaccine Study Group (2005). Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* **191**: 654–665.
26. Jones, NG, DeCamp, A, Gilbert, P, Peterson, ML, Gurwith, M and Cao, H (2009). AIDSVAX immunization induces HIV-specific CD8+ T-cell responses in high-risk, HIV-negative volunteers who subsequently acquire HIV infection. *Vaccine* **27**: 1136–1140.
27. Gilbert, PB, Peterson, ML, Follmann, D, Hudgens, MG, Francis, DP, Gurwith, M *et al.* (2005). Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. *J Infect Dis* **191**: 666–677.
28. Gilbert, PB, Ackers, ML, Berman, PW, Francis, DP, Popovic, V, Hu, DJ *et al.* (2005). HIV-1 virologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. *J Infect Dis* **192**: 974–983.
29. Pitisuttithum, P, Gilbert, P, Gurwith, M, Heyward, W, Martin, M, van Griensven, F *et al.*; Bangkok Vaccine Evaluation Group. (2006). Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* **194**: 1661–1671.
30. Buchbinder, SP, Mehrotra, DV, Duerr, A, Fitzgerald, DW, Mogg, R, Li, D *et al.*; Step Study Protocol Team. (2008). Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* **372**: 1881–1893.
31. McElrath, MJ, De Rosa, SC, Moodie, Z, Dubey, S, Kierstead, L, Janes, H *et al.*; Step Study Protocol Team. (2008). HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* **372**: 1894–1905.
32. Duerr, A, Huang, Y, Buchbinder, S, Coombs, RW, Sanchez, J, del Rio, C *et al.*; Step/HVTN 504 Study Team. (2012). Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). *J Infect Dis* **206**: 258–266.
33. Koblin, BA, Mayer, KH, Noonan, E, Wang, CY, Marmor, M, Sanchez, J *et al.* (2012). Sexual risk behaviors, circumcision status, and preexisting immunity to adenovirus type 5 among men who have sex with men participating in a randomized HIV-1 vaccine efficacy trial: step study. *J Acquir Immune Defic Syndr* **60**: 405–413.
34. Cheng, C Wang, L, Gall, JG, Nason, M, Schwartz, RM, McElrath, MJ, *et al.* (2012). Decreased pre-existing Ad5 capsid and Ad35 neutralizing antibodies increase HIV-1 infection risk in the Step trial independent of vaccination. *PloS one* **7**: e33969.
35. Gray, GE, Allen, M, Moodie, Z, Churchyard, G, Bekker, LG, Nchabeleng, M *et al.*; HVTN 503/Phambili study team. (2011). Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect Dis* **11**: 507–515.
36. Gray, GE, Moodie, Z, Metch, B, Gilbert, PB, Bekker, LG, Churchyard, G *et al.*; HVTN 503/Phambili study team. (2014). Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. *Lancet Infect Dis* **14**: 388–396.
37. Hammer, SM, Sobieszczyk, ME, Janes, H, Karuna, ST, Mulligan, MJ, Grove, D *et al.*; HVTN 505 Study Team. (2013). Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med* **369**: 2083–2092.
38. Rerks-Ngarm, S, Pitisuttithum, P, Nitayaphan, S, Kaewkungwal, J, Chiu, J, Paris, R *et al.*; MOPH-TAVEG Investigators. (2009). Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* **361**: 2209–2220.
39. Gilbert, PB, Berger, JO, Stablein, D, Becker, S, Essex, M, Hammer, SM *et al.* (2011). Statistical interpretation of the RV144 HIV vaccine efficacy trial in Thailand: a case study for statistical issues in efficacy trials. *J Infect Dis* **203**: 969–975.
40. Haynes, BF, Gilbert, PB, McElrath, MJ, Zolla-Pazner, S, Tomaras, GD, Alam, SM *et al.* (2012). Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* **366**: 1275–1286.
41. Montefiori, DC, Karnasuta, C, Huang, Y, Ahmed, H, Gilbert, P, de Souza, MS *et al.* (2012). Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. *J Infect Dis* **206**: 431–441.
42. Rolland, M, Edlefsen, PT, Larsen, BB, Tovanabutra, S, Sanders-Buell, E, Hertz, T *et al.* (2012). Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. *Nature* **490**: 417–420.
43. Karasavvas, N, Billings, E, Rao, M, Williams, C, Zolla-Pazner, S, Bailer, RT *et al.*; MOPH TAVEG Collaboration. (2012). The Thai Phase III HIV Type 1 Vaccine trial (RV144) regimen induces antibodies that target conserved regions within the V2 loop of gp120. *AIDS Res Hum Retroviruses* **28**: 1444–1457.
44. de Souza, MS, Ratto-Kim, S, Chuenarom, W, Schuetz, A, Chantakulkij, S, Nuntapinit, B *et al.*; Ministry of Public Health–Thai AIDS Vaccine Evaluation Group Collaborators. (2012). The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope. *J Immunol* **188**: 5166–5176.
45. Liu, P, Yates, NL, Shen, X, Bonsignori, M, Moody, MA, Liao, HX *et al.* (2013). Infectious virion capture by HIV-1 gp120-specific IgG from RV144 vaccinees. *J Virol* **87**: 7828–7836.
46. Zolla-Pazner, S, deCamp, AC, Cardozo, T, Karasavvas, N, Gottardo, R, Williams, C *et al.* (2013). Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. *PLoS One* **8**: e53629.
47. Pollara, J, Bonsignori, M, Moody, MA, Liu, P, Alam, SM, Hwang, KK *et al.* (2014). HIV-1 vaccine-induced C1 and V2 Env-specific antibodies synergize for increased antiviral activities. *J Virol* **88**: 7715–7726.
48. Johnson, PR, Schnepp, BC, Zhang, J, Connell, MJ, Greene, SM, Yuste, E *et al.* (2009). Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat Med* **15**: 901–906.
49. Fuchs, SP, Martinez-Navio, JM, Piatak, M Jr, Lifson, JD, Gao, G and Desrosiers, RC (2015). AAV-delivered antibody mediates significant protective effects against SIVmac239 challenge in the absence of neutralizing activity. *PLoS Pathog* **11**: e1005090.
50. Gardner, MR, Kattenhorn, LM, Kondur, HR, von Schaewen, M, Dorfman, T, Chiang, JJ *et al.* (2015). AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges. *Nature* **519**: 87–91.
51. Saunders, KO, Wang, L, Joyce, MG, Yang, ZY, Balazs, AB, Cheng, C *et al.* (2015). Broadly neutralizing human immunodeficiency virus type 1 antibody gene transfer protects nonhuman primates from mucosal Simian-human immunodeficiency virus infection. *J Virol* **89**: 8334–8345.
52. Balazs, AB, Chen, J, Hong, CM, Rao, DS, Yang, L and Baltimore, D (2012). Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* **481**: 81–84.
53. Klein, F, Halper-Stromberg, A, Horwitz, JA, Gruell, H, Scheid, JF, Bournazos, S *et al.* (2012). HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* **492**: 118–122.
54. Horwitz, JA, Halper-Stromberg, A, Mouquet, H, Gitlin, AD, Tretiakova, A, Eisenreich, TR, *et al.* (2013). HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc Nat Acad Sci USA* **110**: 16538–16543.
55. Halper-Stromberg, A, Lu, CL, Klein, F, Horwitz, JA, Bournazos, S, Nogueira, L *et al.* (2014). Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. *Cell* **158**: 989–999.
56. Hessell, AJ, Jaworski, JP, Epson, E, Matsuda, K, Pandey, S, Kahl, C *et al.* (2016). Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques. *Nat Med* **22**: 362–368.
57. Barouch, DH, Whitney, JB, Moldt, B, Klein, F, Oliveira, TY, Liu, J *et al.* (2013). Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* **503**: 224–228.
58. Shingai, M, Nishimura, Y, Klein, F, Mouquet, H, Donau, OK, Plishka, R *et al.* (2013). Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* **503**: 277–280.

59. Lynch, RM, Boritz, E, Coates, EE, DeZure, A, Madden, P, Costner, P *et al.*; VRC 601 Study Team. (2015). Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. *Sci Transl Med* **7**: 319ra206.
60. Caskey, M, Klein, F, Lorenzi, JC, Seaman, MS, West, AP Jr, Buckley, N *et al.* (2015). Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* **522**: 487–491.
61. Plotkin, S (2014). History of vaccination. *Proceed Natl Acad Sci USA* **111**: 12283–12287.
62. Plotkin, SA and Plotkin, SL (2011). The development of vaccines: how the past led to the future. *Nat Rev Microbiol* **9**: 889–893.
63. Turner, BG and Summers, MF (1999). Structural biology of HIV. *J Mol Biol* **285**: 1–32.
64. Sierra, S, Kupfer, B and Kaiser, R (2005). Basics of the virology of HIV-1 and its replication. *J Clin Virol* **34**: 233–244.
65. Smyth, RP, Davenport, MP and Mak, J (2012). The origin of genetic diversity in HIV-1. *Virus Res* **169**: 415–429.
66. Hemelaar, J (2012). The origin and diversity of the HIV-1 pandemic. *Trends Mol Med* **18**: 182–192.
67. Maartens, G, Celum, C and Lewin, SR (2014). HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* **384**: 258–271.
68. McMichael, AJ, Borrow, P, Tomaras, GD, Goonetilleke, N and Haynes, BF (2010). The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol* **10**: 11–23.
69. Moir, S, Chun, TW and Fauci, AS (2011). Pathogenic mechanisms of HIV disease. *Annu Rev Pathol* **6**: 223–248.
70. Sharp, PM and Hahn, BH (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med* **1**: a006841.
71. Ho, DD, Neumann, AU, Perelson, AS, Chen, W, Leonard, JM and Markowitz, M (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**: 123–126.
72. Burton, DR (2002). Antibodies, viruses and vaccines. *Nat Rev Immunol* **2**: 706–713.
73. Douek, DC, Brenchley, JM, Betts, MR, Ambrozak, DR, Hill, BJ, Okamoto, Y *et al.* (2002). HIV preferentially infects HIV-specific CD4+ T cells. *Nature* **417**: 95–98.
74. Goulder, PJ and Watkins, DI (2008). Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nat Rev Immunol* **8**: 619–630.
75. McMichael, A and Dorrell, L (2009). The immune response to HIV. *Medicine* **37**: 321–325.
76. Picker, LJ, Hansen, SG and Lifson, JD (2012). New paradigms for HIV/AIDS vaccine development. *Annu Rev Med* **63**: 95–111.
77. Migueles, SA and Connors, M (2015). Success and failure of the cellular immune response against HIV-1. *Nat Immunol* **16**: 563–570.
78. Burton, DR and Mascola, JR (2015). Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat Immunol* **16**: 571–576.
79. Zagury, D, Léonard, R, Fouchard, M, Réveil, B, Bernard, J, Ittelé, D *et al.* (1987). Immunization against AIDS in humans. *Nature* **326**: 249–250.
80. IAVI (2016). IAVI Report - Clinical Trials Database. http://www.iavi.org/trials-database/.
81. Lema, D, Garcia, A and De Sanctis, JB (2014). HIV vaccines: a brief overview. *Scand J Immunol* **80**: 1–11.
82. Benlahrech, A, Harris, J, Meiser, A, Papagatsias, T, Hornig, J, Hayes, P,*et al.* (2009). Adenovirus vector vaccination induces expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1. *Proc Natl Acad Sci USA* **106**: 19940–19945.
83. Pitisuttithum, P, Rerks-Ngarm, S, Bussaratid, V, Dhitavat, J, Maekanantawat, W, Pungpak, S *et al.* (2011). Safety and reactogenicity of canarypox ALVAC-HIV (vCP1521) and HIV-1 gp120 AIDSVAX B/E vaccination in an efficacy trial in Thailand. *PLoS One* **6**: e27837.
84. Goldacre, B (2016). Make journals report clinical trials properly. *Nature* **530**: 7.
85. Edlefsen, PT, Rolland, M, Hertz, T, Tovanabutra, S, Gartland, AJ, deCamp, AC *et al.*; RV144 Sequencing Team. (2015). Comprehensive sieve analysis of breakthrough HIV-1 sequences in the RV144 vaccine efficacy trial. *PLoS Comput Biol* **11**: e1003973.
86. McMichael, AJ and Haynes, BF (2012). Lessons learned from HIV-1 vaccine trials: new priorities and directions. *Nat Immunol* **13**: 423–427.
87. Evans, DT and Silvestri, G (2013). Nonhuman primate models in AIDS research. *Curr Opin HIV AIDS* **8**: 255–261.
88. Hatziioannou, T and Evans, DT (2012). Animal models for HIV/AIDS research. *Nat Rev Microbiol* **10**: 852–867.
89. Watkins, DI, Burton, DR, Kallas, EG, Moore, JP and Koff, WC (2008). Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. *Nat Med* **14**: 617–621.
90. McMichael, A, Picker, LJ, Moore, JP and Burton, DR (2013). Another HIV vaccine failure: where to next? *Nat Med* **19**: 1576–1577.
91. Kestler, H, Kodama, T, Ringler, D, Marthas, M, Pedersen, N, Lackner, A *et al.* (1990). Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* **248**: 1109–1112.
92. Daniel, MD, Letvin, NL, King, NW, Kannagi, M, Sehgal, PK, Hunt, RD *et al.* (1985). Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* **228**: 1201–1204.
93. Harouse, JM, Gettie, A, Eshetu, T, Tan, RC, Bohm, R, Blanchard, J *et al.* (2001). Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). *J Virol* **75**: 1990–1995.
94. Hsu, M, Harouse, JM, Gettie, A, Buckner, C, Blanchard, J and Cheng-Mayer, C (2003). Increased mucosal transmission but not enhanced pathogenicity of the CCR5-tropic, simian AIDS-inducing simian/human immunodeficiency virus SHIV(SF162P3) maps to envelope gp120. *J Virol* **77**: 989–998.
95. Gautam, R, Nishimura, Y, Lee, WR, Donau, O, Buckler-White, A, Shingai, M *et al.* (2012). Pathogenicity and mucosal transmissibility of the R5-tropic simian/human immunodeficiency virus SHIV(AD8) in rhesus macaques: implications for use in vaccine studies. *J Virol* **86**: 8516–8526.
96. Shingai, M , Donau, OK, Schmidt, SD, Gautam, R, Plishka, RJ, Buckler-White, A, *et al.* (2012). Most rhesus macaques infected with the CCR5-tropic SHIV(AD8) generate cross-reactive antibodies that neutralize multiple HIV-1 strains. *Proc Nati Acad Sci USA* **109**: 19769–19774.
97. Koff, WC, Johnson, PR, Watkins, DI, Burton, DR, Lifson, JD, Hasenkrug, KJ *et al.* (2006). HIV vaccine design: insights from live attenuated SIV vaccines. *Nat Immunol* **7**: 19–23.
98. Daniel, MD, Kirchhoff, F, Czajak, SC, Sehgal, PK and Desrosiers, RC (1992). Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science* **258**: 1938–1941.
99. Almond, N, Kent, K, Cranage, M, Rud, E, Clarke, B and Stott, EJ (1995). Protection by attenuated simian immunodeficiency virus in macaques against challenge with virus-infected cells. *Lancet* **345**: 1342–1344.
100. Wyand, MS, Manson, KH, Garcia-Moll, M, Montefiori, D and Desrosiers, RC (1996). Vaccine protection by a triple deletion mutant of simian immunodeficiency virus. *J Virol* **70**: 3724–3733.
101. Norley, S, Beer, B, Binninger-Schinzel, D, Cosma, C and Kurth, R (1996). Protection from pathogenic SIVmac challenge following short-term infection with a nef-deficient attenuated virus. *Virology* **219**: 195–205.
102. Connor, RI, Montefiori, DC, Binley, JM, Moore, JP, Bonhoeffer, S, Gettie, A *et al.* (1998). Temporal analyses of virus replication, immune responses, and efficacy in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. *J Virol* **72**: 7501–7509.
103. Johnson, RP, Lifson, JD, Czajak, SC, Cole, KS, Manson, KH, Glickman, R *et al.* (1999). Highly attenuated vaccine strains of simian immunodeficiency virus protect against vaginal challenge: inverse relationship of degree of protection with level of attenuation. *J Virol* **73**: 4952–4961.
104. Mori, K, Yasutomi, Y, Ohgimoto, S, Nakasone, T, Takamura, S, Shioda, T *et al.* (2001). Quintuple deglycosylation mutant of simian immunodeficiency virus SIVmac239 in rhesus macaques: robust primary replication, tightly contained chronic infection, and elicitation of potent immunity against the parental wild-type strain. *J Virol* **75**: 4023–4028.
105. Manrique, J, Piatak, M, Lauer, W, Johnson, W, Mansfield, K, Lifson, J *et al.* (2013). Influence of mismatch of Env sequences on vaccine protection by live attenuated simian immunodeficiency virus. *J Virol* **87**: 7246–7254.
106. Nilsson, C, Mäkitalo, B, Thorstensson, R, Norley, S, Binninger-Schinzel, D, Cranage, M *et al.* (1998). Live attenuated simian immunodeficiency virus (SIV)mac in macaques can induce protection against mucosal infection with SIVsm. *AIDS* **12**: 2261–2270.
107. Wyand, MS, Manson, K, Montefiori, DC, Lifson, JD, Johnson, RP and Desrosiers, RC (1999). Protection by live, attenuated simian immunodeficiency virus against heterologous challenge. *J Virol* **73**: 8356–8363.
108. Reynolds, MR, Weiler, AM, Weisgrau, KL, Piaskowski, SM, Furlott, JR, Weinfurter, JT *et al.* (2008). Macaques vaccinated with live-attenuated SIV control replication of heterologous virus. *J Exp Med* **205**: 2537–2550.
109. Reynolds, MR, Weiler, AM, Piaskowski, SM, Kolar, HL, Hessell, AJ, Weiker, M *et al.* (2010). Macaques vaccinated with simian immunodeficiency virus SIVmac239Delta nef delay acquisition and control replication after repeated low-dose heterologous SIV challenge. *J Virol* **84**: 9190–9199.
110. Berry, N, Ham, C, Mee, ET, Rose, NJ, Mattiuzzo, G, Jenkins, A *et al.* (2011). Early potent protection against heterologous SIVsmE660 challenge following live attenuated SIV vaccination in Mauritian cynomolgus macaques. *PLoS One* **6**: e23092.
111. Byrareddy, SN, Ayash-Rashkovsky, M, Kramer, VG, Lee, SJ, Correll, M, Novembre, FJ *et al.* (2013). Live attenuated Rev-independent Nef$^-$SIV enhances acquisition of heterologous SIVsmE660 in acutely vaccinated rhesus macaques. *PLoS One* **8**: e75556.
112. Piantadosi, A, Chohan, B, Chohan, V, McClelland, RS and Overbaugh, J (2007). Chronic HIV-1 infection frequently fails to protect against superinfection. *PLoS Pathog* **3**: e177.
113. Jost, S, Bernard, MC, Kaiser, L, Yerly, S, Hirschel, B, Samri, A *et al.* (2002). A patient with HIV-1 superinfection. *N Engl J Med* **347**: 731–736.
114. Altfeld, M, Allen, TM, Yu, XG, Johnston, MN, Agrawal, D, Korber, BT *et al.* (2002). HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. *Nature* **420**: 434–439.
115. Ronen, K, McCoy, CO, Matsen, FA, Boyd, DF, Emery, S, Odem-Davis, K *et al.* (2013). HIV-1 superinfection occurs less frequently than initial infection in a cohort of high-risk Kenyan women. *PLoS Pathog* **9**: e1003593.
116. Blish, CA, Dogan, OC, Jaoko, W, McClelland, RS, Mandaliya, K, Odem-Davis, K *et al.* (2014). Association between cellular immune activation, target cell frequency, and risk of human immunodeficiency virus type 1 superinfection. *J Virol* **88**: 5894–5899.

117. Hansen, SG, Vieville, C, Whizin, N, Coyne-Johnson, L, Siess, DC, Drummond, DD *et al.* (2009). Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med* **15**: 293–299.
118. Hansen, SG, Ford, JC, Lewis, MS, Ventura, AB, Hughes, CM, Coyne-Johnson, L *et al.* (2011). Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* **473**: 523–527.
119. Hansen, SG, Piatak, M Jr, Ventura, AB, Hughes, CM, Gilbride, RM, Ford, JC *et al.* (2013). Immune clearance of highly pathogenic SIV infection. *Nature* **502**: 100–104.
120. Hansen, SG, Sacha, JB, Hughes, CM, Ford, JC, Burwitz, BJ, Scholz, I *et al.* (2013). Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. *Science* **340**: 1237874.
121. Hansen, SG, Wu, HL, Burwitz, BJ, Hughes, CM, Hammond, KB, Ventura, AB *et al.* (2016). Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E. *Science* **351**: 714–720.
122. Hansen, SG, Strelow, LI, Franchi, DC, Anders, DG and Wong, SW (2003). Complete sequence and genomic analysis of rhesus cytomegalovirus. *J Virol* **77**: 6620–6636.
123. Lilja, AE and Shenk, T (2008). Efficient replication of rhesus cytomegalovirus variants in multiple rhesus and human cell types. *Proceed Natl Acad Sci USA* **105**, 19950–19955.
124. Parks, CL, Picker, LJ and King, CR (2013). Development of replication-competent viral vectors for HIV vaccine delivery. *Curr Opin HIV AIDS* **8**: 402–411.
125. Barouch, DH and Picker, LJ (2014). Novel vaccine vectors for HIV-1. *Nat Rev Microbiol* **12**: 765–771.
126. Ondondo, BO (2014). The influence of delivery vectors on HIV vaccine efficacy. *Front Microbiol* **5**: 439.
127. Safrit, JT, Fast, PE, Gieber, L, Kuipers, H, Dean, HJ and Koff, WC (2016). Status of vaccine research and development of vaccines for HIV-1. *Vaccine* **34**: 2921–2925.
128. Barouch, DH, Liu, J, Li, H, Maxfield, LF, Abbink, P, Lynch, DM *et al.* (2012). Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* **482**: 89–93.
129. Barouch, DH, Alter, G, Broge, T, Linde, C, Ackerman, ME, Brown, EP *et al.* (2015). Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. *Science* **349**: 320–324.
130. Baden, LR, Walsh, SR, Seaman, MS, Tucker, RP, Krause, KH, Patel, A *et al.* (2013). First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). *J Infect Dis* **207**: 240–247.
131. Barouch, DH, Liu, J, Peter, L, Abbink, P, Iampietro, MJ, Cheung, A *et al.* (2013). Characterization of humoral and cellular immune responses elicited by a recombinant adenovirus serotype 26 HIV-1 Env vaccine in healthy adults (IPCAVD 001). *J Infect Dis* **207**: 248–256.
132. Baden, LR, Liu, J, Li, H, Johnson, JA, Walsh, SR, Kleinjan, JA *et al.* (2015). Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccination of humans. *J Infect Dis* **211**: 518–528.
133. Bilello, JP, Manrique, JM, Shin, YC, Lauer, W, Li, W, Lifson, JD *et al.* (2011). Vaccine protection against simian immunodeficiency virus in monkeys using recombinant gamma-2 herpesvirus. *J Virol* **85**: 12708–12720.
134. Shin, YC, Bischof, GF, Lauer, WA and Desrosiers, RC (2015). Importance of codon usage for the temporal regulation of viral gene expression. *Proceed Natl Acad Sci USA* **112**: 14030–14035.
135. Sun, C, Chen, Z, Tang, X, Zhang, Y, Feng, L, Du, Y *et al.* (2013). Mucosal priming with a replicating-vaccinia virus-based vaccine elicits protective immunity to simian immunodeficiency virus challenge in rhesus monkeys. *J Virol* **87**: 5669–5677.
136. Kwa, S, Lai, L, Gangadhara, S, Siddiqui, M, Pillai, VB, Labranche, C *et al.* (2014). CD40L-adjuvanted DNA/modified vaccinia virus Ankara simian immunodeficiency virus SIV239 vaccine enhances SIV-specific humoral and cellular immunity and improves protection against a heterologous SIVE660 mucosal challenge. *J Virol* **88**: 9579–9589.
137. Lai, L, Kwa, S, Kozlowski, PA, Montefiori, DC, Ferrari, G, Johnson, WE *et al.* (2011). Prevention of infection by a granulocyte-macrophage colony-stimulating factor co-expressing DNA/modified vaccinia Ankara simian immunodeficiency virus vaccine. *J Infect Dis* **204**: 164–173.
138. Schell, JB, Rose, NF, Bahl, K, Diller, K, Buonocore, L, Hunter, M *et al.* (2011). Significant protection against high-dose simian immunodeficiency virus challenge conferred by a new prime-boost vaccine regimen. *J Virol* **85**: 5764–5772.
139. Weiss, RA, Clapham, PR, Weber, JN, Dalgleish, AG, Lasky, LA and Berman, PW (1986). Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature* **324**: 572–575.
140. Richman, DD, Wrin, T, Little, SJ and Petropoulos, CJ (2003). Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proceed Natl Acad Sci USA* **100**: 4144–4149.
141. Wei, X, Decker, JM, Wang, S, Hui, H, Kappes, JC, Wu, X *et al.* (2003). Antibody neutralization and escape by HIV-1. *Nature* **422**: 307–312.
142. Laird, ME, Igarashi, T, Martin, MA and Desrosiers, RC (2008). Importance of the V1/V2 loop region of simian-human immunodeficiency virus envelope glycoprotein gp120 in determining the strain specificity of the neutralizing antibody response. *J Virol* **82**: 11054–11065.
143. Burns, DP and Desrosiers, RC (1991). Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. *J Virol* **65**: 1843–1854.
144. Burton, DR and Hangartner, L (2016). Broadly neutralizing antibodies to HIV and their role in vaccine design. *Annu Rev Immunol* **34**: 635–659.
145. Sanchez-Merino, V, Fabra-Garcia, A, Gonzalez, N, Nicolas, D, Merino-Mansilla, A, Manzardo, C *et al.* (2016). Detection of broadly neutralizing activity within the first months of HIV-1 infection. *J Virol* **90**: 5231–5245.
146. Doria-Rose, NA, Klein, RM, Manion, MM, O'Dell, S, Phogat, A, Chakrabarti, B *et al.* (2009). Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. *J Virol* **83**: 188–199.
147. Mikell, I, Sather, DN, Kalams, SA, Altfeld, M, Alter, G and Stamatatos, L (2011). Characteristics of the earliest cross-neutralizing antibody response to HIV-1. *PLoS Pathog* **7**: e1001251.
148. Gray, ES, Madiga, MC, Hermanus, T, Moore, PL, Wibmer, CK, Tumba, NL *et al.*; CAPRISA002 Study Team. (2011). The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. *J Virol* **85**: 4828–4840.
149. Klein, F, Mouquet, H, Dosenovic, P, Scheid, JF, Scharf, L and Nussenzweig, MC (2013). Antibodies in HIV-1 vaccine development and therapy. *Science* **341**: 1199–1204.
150. Mouquet, H (2014). Antibody B cell responses in HIV-1 infection. *Trends Immunol* **35**: 549–561.
151. McCoy, LE and Weiss, RA (2013). Neutralizing antibodies to HIV-1 induced by immunization. *J Exp Med* **210**: 209–223.
152. Burton, DR, Desrosiers, RC, Doms, RW, Koff, WC, Kwong, PD, Moore, JP *et al.* (2004). HIV vaccine design and the neutralizing antibody problem. *Nat Immunol* **5**: 233–236.
153. Kepler, TB, Liao, HX, Alam, SM, Bhaskarabhatla, R, Zhang, R, Yandava, C *et al.* (2014). Immunoglobulin gene insertions and deletions in the affinity maturation of HIV-1 broadly reactive neutralizing antibodies. *Cell Host Microbe* **16**: 304–313.
154. Klein, F, Diskin, R, Scheid, JF, Gaebler, C, Mouquet, H, Georgiev, IS *et al.* (2013). Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell* **153**: 126–138.
155. Kwong, PD and Mascola, JR (2012). Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. *Immunity* **37**: 412–425.
156. Sadanand, S, Suscovich, TJ and Alter, G (2016). Broadly neutralizing antibodies against HIV: new insights to inform vaccine design. *Annu Rev Med* **67**: 185–200.
157. Rappuoli, R, Bottomley, MJ, D'Oro, U, Finco, O and De Gregorio, E (2016). Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. *J Exp Med* **213**: 469–481.
158. MacLeod, DT, Choi, NM, Briney, B, Garces, F, Ver, LS, Landais, E *et al.*; IAVI Protocol C Investigators & The IAVI African HIV Research Network. (2016). Early antibody lineage diversification and independent limb maturation lead to broad HIV-1 neutralization targeting the Env high-mannose patch. *Immunity* **44**: 1215–1226.
159. Gorman, J, Soto, C, Yang, MM, Davenport, TM, Guttman, M, Bailer, RT *et al.*; NISC Comparative Sequencing Program. (2016). Structures of HIV-1 Env V1V2 with broadly neutralizing antibodies reveal commonalities that enable vaccine design. *Nat Struct Mol Biol* **23**: 81–90.
160. Bonsignori, M, Zhou, T, Sheng, Z, Chen, L, Gao, F, Joyce, MG *et al.*; NISC Comparative Sequencing Program. (2016). Maturation pathway from germline to broad HIV-1 neutralizer of a CD4-mimic antibody. *Cell* **165**: 449–463.
161. Jardine, JG, Ota, T, Sok, D, Pauthner, M, Kulp, DW, Kalyuzhniy, O *et al.* (2015). HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. *Science* **349**: 156–161.
162. Sanders, RW, van Gils, MJ, Derking, R, Sok, D, Ketas, TJ, Burger, JA *et al.* (2015). HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. *Science* **349**: aac4223.
163. Dosenovic, P, von Boehmer, L, Escolano, A, Jardine, J, Freund, NT, Gitlin, AD *et al.* (2015). Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. *Cell* **161**: 1505–1515.
164. Doria-Rose, NA, Schramm, CA, Gorman, J, Moore, PL, Bhiman, JN, DeKosky, BJ *et al.*; NISC Comparative Sequencing Program. (2014). Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* **509**: 55–62.
165. Georgiou, G, Ippolito, GC, Beausang, J, Busse, CE, Wardemann, H and Quake, SR (2014). The promise and challenge of high-throughput sequencing of the antibody repertoire. *Nat Biotechnol* **32**: 158–168.
166. Liao, HX, Lynch, R, Zhou, T, Gao, F, Alam, SM, Boyd, SD *et al.*; NISC Comparative Sequencing Program. (2013). Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature* **496**: 469–476.
167. Corti, D and Lanzavecchia, A (2013). Broadly neutralizing antiviral antibodies. *Annu Rev Immunol* **31**: 705–742.
168. Haynes, BF, Kelsoe, G, Harrison, SC and Kepler, TB (2012). B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat Biotechnol* **30**: 423–433.
169. Moore, PL, Gray, ES, Wibmer, CK, Bhiman, JN, Nonyane, M, Sheward, DJ *et al.* (2012). Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. *Nat Med* **18**: 1688–1692.

170. Wu, X, Zhou, T, Zhu, J, Zhang, B, Georgiev, I, Wang, C *et al.*; NISC Comparative Sequencing Program. (2011). Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* **333**: 1593–1602.
171. Kwong, PD, Mascola, JR and Nabel, GJ (2013). Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. *Nat Rev Immunol* **13**: 693–701.
172. Shcherbakov, DN, Bakulina, AY, Karpenko, LI and Ilyichev, AA (2015). Broadly neutralizing antibodies against HIV-1 as a novel aspect of the immune response. *Acta Naturae* **7**: 11–21.
173. Burton, DR, Barbas, CF, 3rd, Persson, MA, Koenig, S, Chanock, RM, and Lerner, RA (1991). A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. *Proc Nati Acad Sci USA* **88**: 10134–10137.
174. Barbas, CF 3rd, Bjorling, E, Chiodi, F, Dunlop, N, Cababa, D, Jones, TM, *et al.* (1992). Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus in vitro. *Proc Nati Acad Sci USA* **89**: 9339–9343.
175. Muster, T, Steindl, F, Purtscher, M, Trkola, A, Klima, A, Himmler, G *et al.* (1993). A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. *J Virol* **67**: 6642–6647.
176. Buchacher, A, Predl, R, Strutzenberger, K, Steinfellner, W, Trkola, A, Purtscher, M *et al.* (1994). Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. *AIDS Res Hum Retroviruses* **10**: 359–369.
177. Burton, DR, Pyati, J, Koduri, R, Sharp, SJ, Thornton, GB, Parren, PW *et al.* (1994). Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* **266**: 1024–1027.
178. Muster, T, Guinea, R, Trkola, A, Purtscher, M, Klima, A, Steindl, F *et al.* (1994). Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by the gp41 sequence ELDKWAS. *J Virol* **68**: 4031–4034.
179. Conley, AJ, Kessler, JA, 2nd, Boots, LJ, Tung, JS, Arnold, BA, Keller, PM, *et al.* (1994). Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody. *Proc Natl Acad Sci USA* **91**: 3348–3352.
180. Trkola, A, Purtscher, M, Muster, T, Ballaun, C, Buchacher, A, Sullivan, N *et al.* (1996). Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. *J Virol* **70**: 1100–1108.
181. Zwick, MB, Labrijn, AF, Wang, M, Spenlehauer, C, Saphire, EO, Binley, JM *et al.* (2001). Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. *J Virol* **75**: 10892–10905.
182. Parren, PW, Gauduin, MC, Koup, RA, Poignard, P, Fisicaro, P, Burton, DR *et al.* (1997). Relevance of the antibody response against human immunodeficiency virus type 1 envelope to vaccine design. *Immunol Lett* **57**: 105–112.
183. Walker, LM, Phogat, SK, Chan-Hui, PY, Wagner, D, Phung, P, Goss, JL *et al.*; Protocol G Principal Investigators. (2009). Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* **326**: 285–289.
184. Wu, X, Yang, ZY, Li, Y, Hogerkorp, CM, Schief, WR, Seaman, MS *et al.* (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* **329**: 856–861.
185. Scheid, JF, Mouquet, H, Ueberheide, B, Diskin, R, Klein, F, Oliveira, TY *et al.* (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* **333**: 1633–1637.
186. Klein, F, Gaebler, C, Mouquet, H, Sather, DN, Lehmann, C, Scheid, JF *et al.* (2012). Broad neutralization by a combination of antibodies recognizing the CD4 binding site and a new conformational epitope on the HIV-1 envelope protein. *J Exp Med* **209**: 1469–1479.
187. Walker, LM, Huber, M, Doores, KJ, Falkowska, E, Pejchal, R, Julien, JP *et al.*; Protocol G Principal Investigators. (2011). Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* **477**: 466–470.
188. Pejchal, R, Doores, KJ, Walker, LM, Khayat, R, Huang, PS, Wang, SK *et al.* (2011). A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. *Science* **334**: 1097–1103.
189. Mouquet, H, Scharf, L, Euler, Z, Liu, Y, Eden, C, Scheid, JF, *et al.* (2012). Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc Natl Acad Sci USA* **109**: E3268–3277.
190. Huang, J, Ofek, G, Laub, L, Louder, MK, Doria-Rose, NA, Longo, NS *et al.* (2012). Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature* **491**: 406–412.
191. Kwon, YD, Georgiev, IS, Ofek, G, Zhang, B, Asokan, M, Bailer, RT *et al.* (2016). Optimization of the Solubility of HIV-1-Neutralizing Antibody 10E8 through Somatic Variation and Structure-Based Design. *J Virol* **90**: 5899–5914.
192. Huang, J, Kang, BH, Pancera, M, Lee, JH, Tong, T, Feng, Y *et al.* (2014). Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp120 interface. *Nature* **515**: 138–142.
193. Sok, D, van Gils, MJ, Pauthner, M, Julien, JP, Saye-Francisco, KL, Hsueh, J, *et al.* (2014). Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proc Nati Acad Sci USA* **111**: 17624–17629.
194. Kong, R, Xu, K, Zhou, T, Acharya, P, Lemmin, T, Liu, K *et al.* (2016). Fusion peptide of HIV-1 as a site of vulnerability to neutralizing antibody. *Science* **352**: 828–833.
195. Shibata, R, Igarashi, T, Haigwood, N, Buckler-White, A, Ogert, R, Ross, W *et al.* (1999). Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat Med* **5**: 204–210.
196. Mascola, JR, Lewis, MG, Stiegler, G, Harris, D, VanCott, TC, Hayes, D *et al.* (1999). Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J Virol* **73**: 4009–4018.
197. Mascola, JR, Stiegler, G, VanCott, TC, Katinger, H, Carpenter, CB, Hanson, CE *et al.* (2000). Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat Med* **6**: 207–210.
198. Baba, TW, Liska, V, Hofmann-Lehmann, R, Vlasak, J, Xu, W, Ayehunie, S *et al.* (2000). Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat Med* **6**: 200–206.
199. Parren, PW, Marx, PA, Hessell, AJ, Luckay, A, Harouse, J, Cheng-Mayer, C *et al.* (2001). Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization *in vitro*. *J Virol* **75**: 8340–8347.
200. Hessell, AJ, Poignard, P, Hunter, M, Hangartner, L, Tehrani, DM, Bleeker, WK *et al.* (2009). Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat Med* **15**: 951–954.
201. Hessell, AJ, Rakasz, EG, Poignard, P, Hangartner, L, Landucci, G, Forthal, DN *et al.* (2009). Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog* **5**: e1000433.
202. Hessell, AJ, Rakasz, EG, Tehrani, DM, Huber, M, Weisgrau, KL, Landucci, G *et al.* (2010). Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. *J Virol* **84**: 1302–1313.
203. Burton, DR, Hessell, AJ, Keele, BF, Klasse, PJ, Ketas, TA, Moldt, B, *et al.* (2011). Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc Natl Acad Sci USA* **108**: 11181–11186.
204. Deruaz, M, Moldt, B, Le, KM, Power, KA, Vrbanac, VD, Tanno, S *et al.* (2016). Protection of humanized mice from repeated intravaginal HIV challenge by passive immunization: a model for studying the efficacy of neutralizing antibodies in vivo. *J Infect Dis* **214**: 612–616.
205. Shingai, M, Donau, OK, Plishka, RJ, Buckler-White, A, Mascola, JR, Nabel, GJ *et al.* (2014). Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. *J Exp Med* **211**: 2061–2074.
206. Moldt, B, Rakasz, EG, Schultz, N, Chan-Hui, PY, Swiderek, K, Weisgrau, KL, *et al.* (2012). Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proc Natl Acad Sci USA* **109**: 18921–18925.
207. Ko, SY, Pegu, A, Rudicell, RS, Yang, ZY, Joyce, MG, Chen, X *et al.* (2014). Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* **514**: 642–645.
208. Saunders, KO, Pegu, A, Georgiev, IS, Zeng, M, Joyce, MG, Yang, ZY *et al.* (2015). Sustained delivery of a broadly neutralizing antibody in nonhuman primates confers long-term protection against Simian/human immunodeficiency virus infection. *J Virol* **89**: 5895–5903.
209. Gautam, R, Nishimura, Y, Pegu, A, Nason, MC, Klein, F, Gazumyan, A *et al.* (2016). A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. *Nature* **533**: 105–109.
210. Kong, R, Louder, MK, Wagh, K, Bailer, RT, deCamp, A, Greene, K *et al.* (2015). Improving neutralization potency and breadth by combining broadly reactive HIV-1 antibodies targeting major neutralization epitopes. *J Virol* **89**: 2659–2671.
211. Wagh, K, Bhattacharya, T, Williamson, C, Robles, A, Bayne, M, Garrity, J *et al.* (2016). Optimal combinations of broadly neutralizing antibodies for prevention and treatment of HIV-1 Clade C infection. *PLoS Pathog* **12**: e1005520.
212. Deeks, SG (2012). HIV: shock and kill. *Nature* **487**: 439–440.
213. Hessell, AJ, Hangartner, L, Hunter, M, Havenith, CE, Beurskens, FJ, Bakker, JM *et al.* (2007). Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* **449**: 101–104.
214. Bournazos, S, Klein, F, Pietzsch, J, Seaman, MS, Nussenzweig, MC and Ravetch, JV (2014). Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for *in vivo* activity. *Cell* **158**: 1243–1253.
215. von Bredow, B, Arias, JF, Heyer, LN, Moldt, B, Le, K, Robinson, JE *et al.* (2016). Comparison of antibody-dependent cell-mediated cytotoxicity and virus neutralization by HIV-1 Env-specific monoclonal antibodies. *J Virol* **90**: 6127–6139.
216. Lewis, GK (2014). Role of Fc-mediated antibody function in protective immunity against HIV-1. *Immunology* **142**: 46–57.

217. Bournazos, S, DiLillo, DJ and Ravetch, JV (2015). The role of Fc-FcγR interactions in IgG-mediated microbial neutralization. *J Exp Med* **212**: 1361–1369.
218. Armbruster, C, Stiegler, GM, Vcelar, BA, Jäger, W, Michael, NL, Vetter, N *et al.* (2002). A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. *AIDS* **16**: 227–233.
219. Armbruster, C, Stiegler, GM, Vcelar, BA, Jäger, W, Köller, U, Jilch, R *et al.* (2004). Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. *J Antimicrob Chemother* **54**: 915–920.
220. Stephenson, KE and Barouch, DH (2016). Broadly neutralizing antibodies for HIV eradication. *Curr HIV/AIDS Rep* **13**: 31–37.
221. Stiegler, G, Armbruster, C, Vcelar, B, Stoiber, H, Kunert, R, Michael, NL *et al.* (2002). Antiviral activity of the neutralizing antibodies 2F5 and 2G12 in asymptomatic HIV-1-infected humans: a phase I evaluation. *AIDS* **16**: 2019–2025.
222. Trkola, A, Kuster, H, Rusert, P, Joos, B, Fischer, M, Leemann, C *et al.* (2005). Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat Med* **11**: 615–622.
223. Mehandru, S, Vcelar, B, Wrin, T, Stiegler, G, Joos, B, Mohri, H *et al.* (2007). Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection. *J Virol* **81**: 11016–11031.
224. Ledgerwood, JE, Coates, EE, Yamshchikov, G, Saunders, JG, Holman, L, Enama, ME *et al.*; VRC 602 Study Team. (2015). Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin Exp Immunol* **182**: 289–301.
225. Lu, CL, Murakowski, DK, Bournazos, S, Schoofs, T, Sarkar, D, Halper-Stromberg, A *et al.* (2016). Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* **352**: 1001–1004.
226. Schoofs, T, Klein, F, Braunschweig, M, Kreider, EF, Feldmann, A, Nogueira, L *et al.* (2016). HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* **352**: 997–1001.
227. Scheid, JF, Horwitz, JA, Bar-On, Y, Kreider, EF, Lu, CL, Lorenzi, JC *et al.* (2016). HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* **535**: 556–560.
228. Gorlani, A and Forthal, DN (2013). Antibody-dependent enhancement and the risk of HIV infection. *Curr HIV Res* **11**: 421–426.
229. Mascola, JR, Mathieson, BJ, Zack, PM, Walker, MC, Halstead, SB and Burke, DS (1993). Summary report: workshop on the potential risks of antibody-dependent enhancement in human HIV vaccine trials. *AIDS Res Hum Retroviruses* **9**: 1175–1184.
230. Robinson, WE Jr Montefiori, DC, Mitchell, WM, Prince, AM, Alter, HJ, Dreesman, GR, *et al.* (1989). Antibody-dependent enhancement of human immunodeficiency virus type 1 (HIV-1) infection in vitro by serum from HIV-1-infected and passively immunized chimpanzees. *Proc Nati Acad Sci USA* **86**: 4710–4714.
231. Huang, Y, Yu, J, Lanzi, A, Yao, X, Andrews, CD, Tsai, L *et al.* (2016). Engineered bispecific antibodies with exquisite HIV-1-neutralizing activity. *Cell* **165**: 1621–1631.
232. Bournazos, S, Gazumyan, A, Seaman, MS, Nussenzweig, MC and Ravetch, JV (2016). Bispecific anti-HIV-1 antibodies with enhanced breadth and potency. *Cell* **165**: 1609–1620.
233. Hua, CK and Ackerman, ME (2016). Engineering broadly neutralizing antibodies for HIV prevention and therapy. *Adv Drug Deliv Rev* **103**: 157–173.
234. Sips, M, Krykbaeva, M, Diefenbach, TJ, Ghebremichael, M, Bowman, BA, Dugast, AS, *et al.* (2016). Fc receptor-mediated phagocytosis in tissues as a potent mechanism for preventive and therapeutic HIV vaccine strategies. *Mucosal Immunol* 9: 1584–1595.
235. Sung, JA, Pickeral, J, Liu, L, Stanfield-Oakley, SA, Lam, CY, Garrido, C *et al.* (2015). Dual-affinity re-targeting proteins direct T cell-mediated cytolysis of latently HIV-infected cells. *J Clin Invest* **125**: 4077–4090.
236. Sloan, DD, Lam, CY, Irrinki, A, Liu, L, Tsai, A, Pace, CS *et al.* (2015). Targeting HIV reservoir in infected CD4 T cells by dual-affinity re-targeting molecules (DARTs) that bind HIV envelope and recruit cytotoxic T cells. *PLoS Pathog* **11**: e1005233.
237. Boesch, AW, Alter, G and Ackerman, ME (2015). Prospects for engineering HIV-specific antibodies for enhanced effector function and half-life. *Curr Opin HIV AIDS* **10**: 160–169.
238. Asokan, M, Rudicell, RS, Louder, M, McKee, K, O'Dell, S, Stewart-Jones, G *et al.* (2015). Bispecific antibodies targeting different epitopes on the HIV-1 envelope exhibit broad and potent neutralization. *J Virol* **89**: 12501–12512.
239. Grevys, A, Bern, M, Foss, S, Bratlie, DB, Moen, A, Gunnarsen, KS *et al.* (2015). Fc engineering of human IgG1 for altered binding to the neonatal Fc receptor affects Fc effector functions. *J Immunol* **194**: 5497–5508.
240. Bournazos, S, Chow, SK, Abboud, N, Casadevall, A and Ravetch, JV (2014). Human IgG Fc domain engineering enhances antitoxin neutralizing antibody activity. *J Clin Invest* **124**: 725–729.
241. Romain, G, Senyukov, V, Rey-Villamizar, N, Merouane, A, Kelton, W, Liadi, I *et al.* (2014). Antibody Fc engineering improves frequency and promotes kinetic boosting of serial killing mediated by NK cells. *Blood* **124**: 3241–3249.
242. Mouquet, H, Warncke, M, Scheid, JF, Seaman, MS and Nussenzweig, MC (2012). Enhanced HIV-1 neutralization by antibody heteroligation. *Proc Natl Acad Sci USA* **109**: 875–880.
243. Ackerman, ME, Dugast, AS and Alter, G (2012). Emerging concepts on the role of innate immunity in the prevention and control of HIV infection. *Annu Rev Med* **63**: 113–130.
244. Schaefer, W, Regula, JT, Bahner, M, Schanzer, J, Croasdale, R, Durr, H, *et al.* (2011). Immunoglobulin domain crossover as a generic approach for the production of bispecific IgG antibodies. *Proc Natl Acad Sci USA* **108**: 11187–11192.
245. Moore, GL, Chen, H, Karki, S and Lazar, GA (2010). Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. *MAbs* **2**: 181–189.
246. Strohl, WR (2009). Optimization of Fc-mediated effector functions of monoclonal antibodies. *Curr Opin Biotechnol* **20**: 685–691.
247. Lazar, GA, Dang, W, Karki, S, Vafa, O, Peng, JS, Hyun, L, *et al.* (2006). Engineered antibody Fc variants with enhanced effector function. *Proc Natl Acad Sci USA* **103**: 4005–4010.
248. Kotterman, MA, Chalberg, TW and Schaffer, DV (2015). Viral vectors for gene therapy: translational and clinical outlook. *Annu Rev Biomed Eng* **17**: 63–89.
249. Samulski, RJ and Muzyczka, N (2014). AAV-mediated gene therapy for research and therapeutic purposes. *Annu Rev Virol* **1**: 427–451.
250. Kotterman, MA and Schaffer, DV (2014). Engineering adeno-associated viruses for clinical gene therapy. *Nat Rev Genet* **15**: 445–451.
251. Wang, D, Zhong, L, Nahid, MA and Gao, G (2014). The potential of adeno-associated viral vectors for gene delivery to muscle tissue. *Expert Opin Drug Deliv* **11**: 345–364.
252. Balakrishnan, B and Jayandharan, GR (2014). Basic biology of adeno-associated virus (AAV) vectors used in gene therapy. *Curr Gene Ther* **14**: 86–100.
253. Mingozzi, F and High, KA (2011). Therapeutic *in vivo* gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet* **12**: 341–355.
254. Daya, S and Berns, KI (2008). Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev* **21**: 583–593.
255. Gil-Farina, I, Fronza, R, Kaeppel, C, Lopez-Franco, E, Ferreira, V, D'Avola, D *et al.* (2016). Recombinant AAV integration is not associated with hepatic genotoxicity in nonhuman primates and patients. *Mol Ther* **24**: 1100–1105.
256. Berns, KI, Byrne, BJ, Flotte, TR, Gao, G, Hauswirth, WW, Herzog, RW *et al.* (2015). Adeno-associated virus type 2 and hepatocellular carcinoma? *Hum Gene Ther* **26**: 779–781.
257. Kaeppel, C, Beattie, SG, Fronza, R, van Logtenstein, R, Salmon, F, Schmidt, S *et al.* (2013). A largely random AAV integration profile after LPLD gene therapy. *Nat Med* **19**: 889–891.
258. Ye, GJ, Budzynski, E, Sonnentag, P, Nork, TM, Miller, PE, Sharma, AK *et al.* (2016). Safety and biodistribution evaluation in Cynomolgus Macaques of rAAV2tYF-PR1.7-hCNGB3, a recombinant AAV vector for treatment of achromatopsia. *Hum Gene Ther Clin Dev* **27**: 37–48.
259. Mancuso, K, Hauswirth, WW, Li, Q, Connor, TB, Kuchenbecker, JA, Mauck, MC *et al.* (2009). Gene therapy for red-green colour blindness in adult primates. *Nature* **461**: 784–787.
260. Nathwani, AC, Gray, JT, McIntosh, J, Ng, CY, Zhou, J, Spence, Y *et al.* (2007). Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. *Blood* **109**: 1414–1421.
261. Rivera, VM, Gao, GP, Grant, RL, Schnell, MA, Zoltick, PW, Rozamus, LW *et al.* (2005). Long-term pharmacologically regulated expression of erythropoietin in primates following AAV-mediated gene transfer. *Blood* **105**: 1424–1430.
262. Manno, CS, Chew, AJ, Hutchison, S, Larson, PJ, Herzog, RW, Arruda, VR *et al.* (2003). AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. *Blood* **101**: 2963–2972.
263. Manno, CS, Pierce, GF, Arruda, VR, Glader, B, Ragni, M, Rasko, JJ *et al.* (2006). Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* **12**: 342–347.
264. Nathwani, AC, Tuddenham, EG, Rangarajan, S, Rosales, C, McIntosh, J, Linch, DC *et al.* (2011). Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* **365**: 2357–2365.
265. Nathwani, AC, Reiss, UM, Tuddenham, EG, Rosales, C, Chowdary, P, McIntosh, J *et al.* (2014). Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med* **371**: 1994–2004.
266. Stroes, ES, Nierman, MC, Meulenberg, JJ, Franssen, R, Twisk, J, Henny, CP *et al.* (2008). Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol* **28**: 2303–2304.
267. Gaudet, D, Méthot, J, Déry, S, Brisson, D, Essiembre, C, Tremblay, G *et al.* (2013). Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for lipoprotein lipase deficiency: an open-label trial. *Gene Ther* **20**: 361–369.
268. Brantly, ML, Chulay, JD, Wang, L, Mueller, C, Humphries, M, Spencer, LT, *et al.* (2009). Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. *Proc Natil Acad Sci USA* **106**: 16363–16368.
269. Flotte, TR, Trapnell, BC, Humphries, M, Carey, B, Calcedo, R, Rouhani, F *et al.* (2011). Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing α1-antitrypsin: interim results. *Hum Gene Ther* **22**: 1239–1247.

270. MacLaren, RE, Groppe, M, Barnard, AR, Cottriall, CL, Tolmachova, T, Seymour, L *et al.* (2014). Retinal gene therapy in patients with choroideremia: initial findings from a phase ½ clinical trial. *Lancet* **383**: 1129–1137.
271. Bowles, DE, McPhee, SW, Li, C, Gray, SJ, Samulski, JJ, Camp, AS *et al.* (2012). Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. *Mol Ther* **20**: 443–455.
272. Maguire, AM, Simonelli, F, Pierce, EA, Pugh, EN Jr, Mingozzi, F, Bennicelli, J *et al.* (2008). Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* **358**: 2240–2248.
273. Jacobson, SG, Cideciyan, AV, Ratnakaram, R, Heon, E, Schwartz, SB, Roman, AJ *et al.* (2012). Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch Ophthalmol* **130**: 9–24.
274. LeWitt, PA, Rezai, AR, Leehey, MA, Ojemann, SG, Flaherty, AW, Eskandar, EN *et al.* (2011). AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol* **10**: 309–319.
275. Bennett, J, Ashtari, M, Wellman, J, Marshall, KA, Cyckowski, LL, Chung, DC *et al.* (2012). AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med* **4**: 120ra15.
276. Ylä-Herttuala, S (2012). Endgame: glybera finally recommended for approval as the first gene therapy drug in the European union. *Mol Ther* **20**: 1831–1832.
277. Bryant, LM, Christopher, DM, Giles, AR, Hinderer, C, Rodriguez, JL, Smith, JB *et al.* (2013). Lessons learned from the clinical development and market authorization of Glybera. *Hum Gene Ther Clin Dev* **24**: 55–64.
278. Scott, LJ (2015). Alipogene tiparvovec: a review of its use in adults with familial lipoprotein lipase deficiency. *Drugs* **75**: 175–182.
279. Kastelein, JJ, Ross, CJ and Hayden, MR (2013). From mutation identification to therapy: discovery and origins of the first approved gene therapy in the Western world. *Hum Gene Ther* **24**: 472–478.
280. Hermonat, PL and Muzyczka, N (1984). Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells. *Proc Natl Acad Sci USA* **81**: 6466–6470.
281. McLaughlin, SK, Collis, P, Hermonat, PL and Muzyczka, N (1988). Adeno-associated virus general transduction vectors: analysis of proviral structures. *J Virol* **62**: 1963–1973.
282. Samulski, RJ, Chang, LS and Shenk, T (1989). Helper-free stocks of recombinant adeno-associated viruses: normal integration does not require viral gene expression. *J Virol* **63**: 3822–3828.
283. Gonçalves, MA (2005). Adeno-associated virus: from defective virus to effective vector. *Virol J* **2**: 43.
284. Le Bec, C and Douar, AM (2006). Gene therapy progress and prospects–vectorology: design and production of expression cassettes in AAV vectors. *Gene Ther* **13**: 805–813.
285. Schnepp, BC, Clark, KR, Klemanski, DL, Pacak, CA and Johnson, PR (2003). Genetic fate of recombinant adeno-associated virus vector genomes in muscle. *J Virol* **77**: 3495–3504.
286. Schnepp, BC, Chulay, JD, Ye, GJ, Flotte, TR, Trapnell, BC and Johnson, PR (2016). Recombinant adeno-associated virus vector genomes take the form of long-lived, transcriptionally competent episomes in human muscle. *Hum Gene Ther* **27**: 32–42.
287. Flotte, TR, , Mueller, C, Gernoux, G, Gruntman, A, Chulay, JD, Knop, DR, *et al*. (2016). Sustained Expression with Partial Correction of Neutrophil Defects 5 Years After Intramuscular rAAV1 Gene Therapy for Alpha-1 Antitrypsin Deficiency. http://escholarship.umassmed.edu/cgi/viewcontent.cgi?article=1385&context=cts_retreat.
288. Saunders, KO, Wang, L, Joyce, MG, Yang, ZY, Balazs, AB, Cheng, C *et al.* (2015). Broadly neutralizing human immunodeficiency virus type 1 antibody gene transfer protects nonhuman primates from mucosal Simian-human immunodeficiency virus infection. *J Virol* **89**: 8334–8345.
289. Mellins, ED and Kay, MA (2015). Viral vectors take on HIV infection. *N Engl J Med* **373**: 770–772.
290. Muzyczka, N and Berns, KI (2015). AAV's Golden Jubilee. *Mol Ther* **23**: 807–808.
291. Balazs, AB, Ouyang, Y, Hong, CM, Chen, J, Nguyen, SM, Rao, DS *et al.* (2014). Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. *Nat Med* **20**: 296–300.
292. McCarty, DM (2008). Self-complementary AAV vectors; advances and applications. *Mol Ther* **16**: 1648–1656.
293. McCarty, DM, Monahan, PE and Samulski, RJ (2001). Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther* **8**: 1248–1254.
294. Wu, Z, Yang, H and Colosi, P (2010). Effect of genome size on AAV vector packaging. *Mol Ther* **18**: 80–86.
295. Wu, J, Zhao, W, Zhong, L, Han, Z, Li, B, Ma, W *et al.* (2007). Self-complementary recombinant adeno-associated viral vectors: packaging capacity and the role of rep proteins in vector purity. *Hum Gene Ther* **18**: 171–182.
296. Fuchs, SP, Martinez-Navio, JM, Gao, G and Desrosiers, RC (2016). Recombinant AAV vectors for enhanced expression of authentic IgG. *PLoS One* **11**: e0158009.
297. Fang, J, Qian, JJ, Yi, S, Harding, TC, Tu, GH, VanRoey, M *et al.* (2005). Stable antibody expression at therapeutic levels using the 2A peptide. *Nat Biotechnol* **23**: 584–590.
298. Fang, J, Yi, S, Simmons, A, Tu, GH, Nguyen, M, Harding, TC *et al.* (2007). An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo. *Mol Ther* **15**: 1153–1159.
299. Choe, H, Li, W, Wright, PL, Vasilieva, N, Venturi, M, Huang, CC *et al.* (2003). Tyrosine sulfation of human antibodies contributes to recognition of the CCR5 binding region of HIV-1 gp120. *Cell* **114**: 161–170.
300. Dorfman, T, Moore, MJ, Guth, AC, Choe, H and Farzan, M (2006). A tyrosine-sulfated peptide derived from the heavy-chain CDR3 region of an HIV-1-neutralizing antibody binds gp120 and inhibits HIV-1 infection. *J Biol Chem* **281**: 28529–28535.
301. Chiang, JJ, Gardner, MR, Quinlan, BD, Dorfman, T, Choe, H and Farzan, M (2012). Enhanced recognition and neutralization of HIV-1 by antibody-derived CCR5-mimetic peptide variants. *J Virol* **86**: 12417–12421.
302. Gardner, MR, Fellinger, CH, Prasad, NR, Zhou, AS, Kondur, HR, Joshi, VR *et al.* (2016). CD4-induced antibodies promote association of the HIV-1 envelope glycoprotein with CD4-binding site antibodies. *J Virol* **90**: 7822–7832.
303. Mingozzi, F and Büning, H (2015). Adeno-associated viral vectors at the frontier between tolerance and immunity. *Front Immunol* **6**: 120.
304. Mingozzi, F and High, KA (2013). Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood* **122**: 23–36.
305. Hareendran, S, Balakrishnan, B, Sen, D, Kumar, S, Srivastava, A and Jayandharan, GR (2013). Adeno-associated virus (AAV) vectors in gene therapy: immune challenges and strategies to circumvent them. *Rev Med Virol* **23**: 399–413.
306. Ferreira, V, Petry, H and Salmon, F (2014). Immune responses to AAV-vectors, the Glybera example from bench to bedside. *Front Immunol* **5**: 82.
307. Rogers, GL, Martino, AT, Aslanidi, GV, Jayandharan, GR, Srivastava, A and Herzog, RW (2011). Innate immune responses to AAV vectors. *Front Microbiol* **2**: 194.
308. Basner-Tschakarjan, E and Mingozzi, F (2014). Cell-mediated immunity to AAV vectors, evolving concepts and potential solutions. *Front Immunol* **5**: 350.
309. Calcedo, R and Wilson, JM (2013). Humoral immune response to AAV. *Front Immunol* **4**: 341.
310. Veron, P, Leborgne, C, Monteilhet, V, Boutin, S, Martin, S, Moullier, P *et al.* (2012). Humoral and cellular capsid-specific immune responses to adeno-associated virus type 1 in randomized healthy donors. *J Immunol* **188**: 6418–6424.
311. Gao, G, Vandenberghe, LH and Wilson, JM (2005). New recombinant serotypes of AAV vectors. *Curr Gene Ther* **5**: 285–297.
312. Gao, G, Vandenberghe, LH, Alvira, MR, Lu, Y, Calcedo, R, Zhou, X *et al.* (2004). Clades of adeno-associated viruses are widely disseminated in human tissues. *J Virol* **78**: 6381–6388.
313. Gao, GP, Alvira, MR, Wang, L, Calcedo, R, Johnston, J, and Wilson, JM (2002). Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *Proc Nati Acad Sci USA* **99**: 11854–11859.
314. Hösel, M, Broxtermann, M, Janicki, H, Esser, K, Arzberger, S, Hartmann, P *et al.* (2012). Toll-like receptor 2-mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors. *Hepatology* **55**: 287–297.
315. Zhu, J, Huang, X and Yang, Y (2009). The TLR9-MyD88 pathway is critical for adaptive immune responses to adeno-associated virus gene therapy vectors in mice. *J Clin Invest* **119**: 2388–2398.
316. Martino, AT, Suzuki, M, Markusic, DM, Zolotukhin, I, Ryals, RC, Moghimi, B *et al.* (2011). The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9-dependent innate immune responses in the liver. *Blood* **117**: 6459–6468.
317. Faust, SM, Bell, P, Cutler, BJ, Ashley, SN, Zhu, Y, Rabinowitz, JE *et al.* (2013). CpG-depleted adeno-associated virus vectors evade immune detection. *J Clin Invest* **123**: 2994–3001.
318. Sudres, M, Ciré, S, Vasseur, V, Brault, L, Da Rocha, S, Boisgérault, F *et al.* (2012). MyD88 signaling in B cells regulates the production of Th1-dependent antibodies to AAV. *Mol Ther* **20**: 1571–1581.
319. Calcedo, R, Vandenberghe, LH, Gao, G, Lin, J and Wilson, JM (2009). Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. *J Infect Dis* **199**: 381–390.
320. Boutin, S, Monteilhet, V, Veron, P, Leborgne, C, Benveniste, O, Montus, MF *et al.* (2010). Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Hum Gene Ther* **21**: 704–712.
321. Calcedo, R and Wilson, JM (2016). AAV Natural Infection Induces Broad Cross-Neutralizing Antibody Responses to Multiple AAV Serotypes in Chimpanzees. *Hum Gene Ther Clin Dev* **27**: 79–82.
322. Scallan, CD, Jiang, H, Liu, T, Patarroyo-White, S, Sommer, JM, Zhou, S *et al.* (2006). Human immunoglobulin inhibits liver transduction by AAV vectors at low AAV2 neutralizing titers in SCID mice. *Blood* **107**: 1810–1817.
323. Jiang, H, Couto, LB, Patarroyo-White, S, Liu, T, Nagy, D, Vargas, JA *et al.* (2006). Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood* **108**: 3321–3328.

324. Murphy, SL, Li, H, Zhou, S, Schlachterman, A, High, KA and High, K (2008). Prolonged susceptibility to antibody-mediated neutralization for adeno-associated vectors targeted to the liver. *Mol Ther* **16**: 138–145.
325. Fisher, KJ, Jooss, K, Alston, J, Yang, Y, Haecker, SE, High, K *et al.* (1997). Recombinant adeno-associated virus for muscle directed gene therapy. *Nat Med* **3**: 306–312.
326. Rivière, C, Danos, O and Douar, AM (2006). Long-term expression and repeated administration of AAV type 1, 2 and 5 vectors in skeletal muscle of immunocompetent adult mice. *Gene Ther* **13**: 1300–1308.
327. Petry, H, Brooks, A, Orme, A, Wang, P, Liu, P, Xie, J *et al.* (2008). Effect of viral dose on neutralizing antibody response and transgene expression after AAV1 vector re-administration in mice. *Gene Ther* **15**: 54–60.
328. Manning, WC, Zhou, S, Bland, MP, Escobedo, JA and Dwarki, V (1998). Transient immunosuppression allows transgene expression following readministration of adeno-associated viral vectors. *Hum Gene Ther* **9**: 477–485.
329. Halbert, CL, Standaert, TA, Wilson, CB and Miller, AD (1998). Successful readministration of adeno-associated virus vectors to the mouse lung requires transient immunosuppression during the initial exposure. *J Virol* **72**: 9795–9805.
330. Chirmule, N, Xiao, W, Truneh, A, Schnell, MA, Hughes, JV, Zoltick, P *et al.* (2000). Humoral immunity to adeno-associated virus type 2 vectors following administration to murine and nonhuman primate muscle. *J Virol* **74**: 2420–2425.
331. Pien, GC, Basner-Tschakarjan, E, Hui, DJ, Mentlik, AN, Finn, JD, Hasbrouck, NC *et al.* (2009). Capsid antigen presentation flags human hepatocytes for destruction after transduction by adeno-associated viral vectors. *J Clin Invest* **119**: 1688–1695.
332. Rogers, GL, Martino, AT, Zolotukhin, I, Ertl, HC and Herzog, RW (2014). Role of the vector genome and underlying factor IX mutation in immune responses to AAV gene therapy for hemophilia B. *J Transl Med* **12**: 25.
333. Cao, O, Hoffman, BE, Moghimi, B, Nayak, S, Cooper, M, Zhou, S *et al.* (2009). Impact of the underlying mutation and the route of vector administration on immune responses to factor IX in gene therapy for hemophilia B. *Mol Ther* **17**: 1733–1742.
334. Fields, PA, Arruda, VR, Armstrong, E, Chu, K, Mingozzi, F, Hagstrom, JN *et al.* (2001). Risk and prevention of anti-factor IX formation in AAV-mediated gene transfer in the context of a large deletion of F9. *Mol Ther* **4**: 201–210.
335. Herzog, RW, Hagstrom, JN, Kung, SH, Tai, SJ, Wilson, JM, Fisher, KJ, *et al.* (1997). Stable gene transfer and expression of human blood coagulation factor IX after intramuscular injection of recombinant adeno-associated virus. *Proc Natl Acad Sci USA* **94**: 5804–5809.
336. Herzog, RW, Yang, EY, Couto, LB, Hagstrom, JN, Elwell, D, Fields, PA *et al.* (1999). Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. *Nat Med* **5**: 56–63.
337. Mendell, JR, Campbell, K, Rodino-Klapac, L, Sahenk, Z, Shilling, C, Lewis, S *et al.* (2010). Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med* **363**: 1429–1437.
338. Li, C, Goudy, K, Hirsch, M, Asokan, A, Fan, Y, Alexander, J,*et al.* (2009). Cellular immune response to cryptic epitopes during therapeutic gene transfer. *Proc Natl Acad Sci USA* **106**: 10770–10774.
339. Markusic, DM, Hoffman, BE, Perrin, GQ, Nayak, S, Wang, X, LoDuca, PA *et al.* (2013). Effective gene therapy for haemophilic mice with pathogenic factor IX antibodies. *EMBO Mol Med* **5**: 1698–1709.
340. Mays, LE, Vandenberghe, LH, Xiao, R, Bell, P, Nam, HJ, Agbandje-McKenna, M *et al.* (2009). Adeno-associated virus capsid structure drives CD4-dependent CD8+ T cell response to vector encoded proteins. *J Immunol* **182**: 6051–6060.
341. Mays, LE and Wilson, JM (2011). The complex and evolving story of T cell activation to AAV vector-encoded transgene products. *Mol Ther* **19**: 16–27.
342. Lu, Y and Song, S (2009). Distinct immune responses to transgene products from rAAV1 and rAAV8 vectors. *Proc Natl Acad Sci USA* **106**: 17158–17162.
343. Sen, D, Gadkari, RA, Sudha, G, Gabriel, N, Kumar, YS, Selot, R *et al.* (2013). Targeted modifications in adeno-associated virus serotype 8 capsid improves its hepatic gene transfer efficiency in vivo. *Hum Gene Ther Methods* **24**: 104–116.
344. Wang, L, Louboutin, JP, Bell, P, Greig, JA, Li, Y, Wu, D *et al.* (2011). Muscle-directed gene therapy for hemophilia B with more efficient and less immunogenic AAV vectors. *J Thromb Haemost* **9**: 2009–2019.
345. Wu, T, Töpfer, K, Lin, SW, Li, H, Bian, A, Zhou, XY *et al.* (2012). Self-complementary AAVs induce more potent transgene product-specific immune responses compared to a single-stranded genome. *Mol Ther* **20**: 572–579.
346. Greig, JA, Peng, H, Ohlstein, J, Medina-Jaszek, CA, Ahonkhai, O, Mentzinger, A *et al.* (2014). Intramuscular injection of AAV8 in mice and macaques is associated with substantial hepatic targeting and transgene expression. *PLoS One* **9**: e112268.
347. Li, C, Diprimio, N, Bowles, DE, Hirsch, ML, Monahan, PE, Asokan, A *et al.* (2012). Single amino acid modification of adeno-associated virus capsid changes transduction and humoral immune profiles. *J Virol* **86**: 7752–7759.
348. Toromanoff, A, Adjali, O, Larcher, T, Hill, M, Guigand, L, Chenuaud, P *et al.* (2010). Lack of immunotoxicity after regional intravenous (RI) delivery of rAAV to nonhuman primate skeletal muscle. *Mol Ther* **18**: 151–160.
349. Xin, KQ, Mizukami, H, Urabe, M, Toda, Y, Shinoda, K, Yoshida, A *et al.* (2006). Induction of robust immune responses against human immunodeficiency virus is supported by the inherent tropism of adeno-associated virus type 5 for dendritic cells. *J Virol* **80**: 11899–11910.
350. Veron, P, Allo, V, Rivière, C, Bernard, J, Douar, AM and Masurier, C (2007). Major subsets of human dendritic cells are efficiently transduced by self-complementary adeno-associated virus vectors 1 and 2. *J Virol* **81**: 5385–5394.
351. Schroeder, HW Jr and Cavacini, L (2010). Structure and function of immunoglobulins. *J Allergy Clin Immunol* **125**(2 Suppl 2): S41–S52.
352. Mix, E, Goertsches, R and Zett, UK (2006). Immunoglobulins–basic considerations. *J Neurol* **253 Suppl 5**: V9–17.
353. Melchers, F (2015). Checkpoints that control B cell development. *J Clin Invest* **125**: 2203–2210.
354. Wardemann, H, Yurasov, S, Schaefer, A, Young, JW, Meffre, E and Nussenzweig, MC (2003). Predominant autoantibody production by early human B cell precursors. *Science* **301**: 1374–1377.
355. Wardemann, H and Nussenzweig, MC (2007). B-cell self-tolerance in humans. *Adv Immunol* **95**: 83–110.
356. Mouquet, H and Nussenzweig, MC (2012). Polyreactive antibodies in adaptive immune responses to viruses. *Cell Mol Life Sci* **69**: 1435–1445.
357. Sok, D, Laserson, U, Laserson, J, Liu, Y, Vigneault, F, Julien, JP *et al.* (2013). The effects of somatic hypermutation on neutralization and binding in the PGT121 family of broadly neutralizing HIV antibodies. *PLoS Pathog* **9**: e1003754.
358. Mouquet, H, Scheid, JF, Zoller, MJ, Krogsgaard, M, Ott, RG, Shukair, S *et al.* (2010). Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. *Nature* **467**: 591–595.
359. Martinez-Navio, JM, Fuchs, SP, Pedreño-López, S, Rakasz, EG, Gao, G and Desrosiers, RC (2016). Host anti-antibody responses following adeno-associated virus-mediated delivery of antibodies against HIV and SIV in Rhesus monkeys. *Mol Ther* **24**: 76–86.
360. Breden, F, Lepik, C, Longo, NS, Montero, M, Lipsky, PE and Scott, JK (2011). Comparison of antibody repertoires produced by HIV-1 infection, other chronic and acute infections, and systemic autoimmune disease. *PLoS One* **6**: e16857.
361. Harding, FA, Stickler, MM, Razo, J and DuBridge, RB (2010). The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs* **2**: 256–265.
362. Krishna, M and Nadler, SG (2016). Immunogenicity to biotherapeutics - the role of anti-drug immune complexes. *Front Immunol* **7**: 21.
363. Scott, DW and De Groot, AS (2010). Can we prevent immunogenicity of human protein drugs? *Ann Rheum Dis* **69 Suppl 1**: i72–i76.
364. van Beers, MM and Bardor, M (2012). Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. *Biotechnol J* **7**: 1473–1484.
365. Baker, MP, Reynolds, HM, Lumicisi, B and Bryson, CJ (2010). Immunogenicity of protein therapeutics: the key causes, consequences and challenges. *Self Nonself* **1**: 314–322.
366. Bartelds, GM, Wolbink, GJ, Stapel, S, Aarden, L, Lems, WF, Dijkmans, BA *et al.* (2006). High levels of human anti-human antibodies to adalimumab in a patient not responding to adalimumab treatment. *Ann Rheum Dis* **65**: 1249–1250.
367. Wang, W, Wang, EQ and Balthasar, JP (2008). Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* **84**: 548–558.
368. Radstake, TR, Svenson, M, Eijsbouts, AM, van den Hoogen, FH, Enevold, C, van Riel, PL *et al.* (2009). Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* **68**: 1739–1745.
369. van Meer, PJ, Kooijman, M, Brinks, V, Gispen-de Wied, CC, Silva-Lima, B, Moors, EH *et al.* (2013). Immunogenicity of mAbs in non-human primates during nonclinical safety assessment. *MAbs* **5**: 810–816.
370. Hwang, WY and Foote, J (2005). Immunogenicity of engineered antibodies. *Methods* **36**: 3–10.
371. Thullier, P, Chahboun, S and Pelat, T (2010). A comparison of human and macaque (Macaca mulatta) immunoglobulin germline V regions and its implications for antibody engineering. *MAbs* **2**: 528–538.
372. West, AP Jr, Galimidi, RP, Gnanapragasam, PN and Bjorkman, PJ (2012). Single-chain Fv-based anti-HIV proteins: potential and limitations. *J Virol* **86**: 195–202.
373. Rudicell, RS, Kwon, YD, Ko, SY, Pegu, A, Louder, MK, Georgiev, IS *et al.*; NISC Comparative Sequencing Program. (2014). Enhanced potency of a broadly neutralizing HIV-1 antibody *in vitro* improves protection against lentiviral infection in vivo. *J Virol* **88**: 12669–12682.
374. Corti, M, Elder, M, Falk, D, Lawson, L, Smith, B, Nayak, S *et al.* (2014). B-Cell Depletion is Protective Against Anti-AAV Capsid Immune Response: A Human Subject Case Study. *Mol Ther Methods Clin Dev* **1**: 14033; doi:14010.11038/mtm.12014.14033.
375. Wagner, CL, Schantz, A, Barnathan, E, Olson, A, Mascelli, MA, Ford, J *et al.* (2003). Consequences of immunogenicity to the therapeutic monoclonal antibodies ReoPro and Remicade. *Dev Biol (Basel)* **112**: 37–53.
376. Zhong, L, Li, B, Mah, CS, Govindasamy, L, Agbandje-McKenna, M, Cooper, M, *et al.* (2008). Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. *Proc Natl Acad Sci USA* **105**: 7827–7832.

377. Qiao, C, Zhang, W, Yuan, Z, Shin, JH, Li, J, Jayandharan, GR *et al.* (2010). Adeno-associated virus serotype 6 capsid tyrosine-to-phenylalanine mutations improve gene transfer to skeletal muscle. *Hum Gene Ther* **21**: 1343–1348.
378. Vercauteren, K, Hoffman, BE, Zolotukhin, I, Keeler, GD, Xiao, JW, Basner-Tschakarjan, E *et al.* (2016). Superior *in vivo* transduction of human hepatocytes using engineered AAV3 capsid. *Mol Ther* **24**: 1042–1049.
379. Majowicz, A, Maczuga, P, Kwikkers, KL, van der Marel, S, van Logtenstein, R, Petry, H *et al.* (2013). Mir-142-3p target sequences reduce transgene-directed immunogenicity following intramuscular adeno-associated virus 1 vector-mediated gene delivery. *J Gene Med* **15**: 219–232.
380. Boisgerault, F, Gross, DA, Ferrand, M, Poupiot, J, Darocha, S, Richard, I *et al.* (2013). Prolonged gene expression in muscle is achieved without active immune tolerance using microrRNA 142.3p-regulated rAAV gene transfer. *Hum Gene Ther* **24**: 393–405.
381. Wang, X, Terhorst, C and Herzog, RW (2016). *In vivo* induction of regulatory T cells for immune tolerance in hemophilia. *Cell Immunol* **301**: 18–29.
382. Sharland, A, Logan, GJ, Bishop, A and Alexander, IE (2010). Liver-directed gene expression using recombinant AAV 2/8 vectors–a tolerogenic strategy for gene delivery? *Discov Med* **9**: 519–527.
383. Sack, BK, Herzog, RW, Terhorst, C and Markusic, DM (2014). Development of gene transfer for induction of antigen-specific tolerance. *Mol Ther Methods Clin Dev* **1**: 14013.
384. Mueller, C, Chulay, JD, Trapnell, BC, Humphries, M, Carey, B, Sandhaus, RA *et al.* (2013). Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. *J Clin Invest* **123**: 5310–5318.
385. Mays, LE, Wang, L, Lin, J, Bell, P, Crawford, A, Wherry, EJ *et al.* (2014). AAV8 induces tolerance in murine muscle as a result of poor APC transduction, T cell exhaustion, and minimal MHCI upregulation on target cells. *Mol Ther* **22**: 28–41.
386. LoDuca, PA, Hoffman, BE and Herzog, RW (2009). Hepatic gene transfer as a means of tolerance induction to transgene products. *Curr Gene Ther* **9**: 104–114.
387. Büning, H and Schmidt, M (2015). Adeno-associated vector toxicity-to be or not to be? *Mol Ther* **23**: 1673–1675.