

Review

HIV DNA Vaccine: Stepwise Improvements Make a Difference

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Abstract: Inefficient DNA delivery methods and low expression of plasmid DNA have been major obstacles for the use of plasmid DNA as vaccine for HIV/AIDS. This review describes successful efforts to improve DNA vaccine methodology over the past ~30 years. DNA vaccination, either alone or in combination with other methods, has the potential to be a rapid, safe, and effective vaccine platform against AIDS. Recent clinical trials suggest the feasibility of its translation to the clinic.

Keywords: macaque; cytokine; plasmid; electroporation; immunogenicity; vaccination; DNA delivery; DNA expression

1. Introduction

Different vaccine approaches, including the use of plasmid DNA, recombinant viral vectors, protein or peptides and combination thereof in prime-boost regimens are being pursued as potential vaccines against HIV/AIDS. More than 30 trials are currently being conducted to evaluate the immunogenicity of promising HIV vaccine candidates. Four efficacy clinical trials have been completed: (i) gp120 (VaxGen) [1–4]; (ii) recombinant Ad5 (STEP) [5–7]; (iii) DNA prime-recombinant Ad5 boost

(HVTN 505) [8]; (IV) combination of recombinant Canarypox ALVAC[®]-HIV (vCP1521; containing Gag, PR and Env) with gp120 Env protein (AIDSVAX[®] B/E) [9]. Although the first three trials failed to show efficacy, the latter resulted in modest statistically significant protection from infection in the RV144 vaccine trial conducted in Thailand [9]. The limited efficacy and the fact that the vaccine-induced responses waned over time indicate that improved vaccine designs are needed to achieve long-lasting cross-clade immune responses able to prevent and contain infection. Importantly, the RV144 trial revealed a critical role of humoral responses in preventing infection. Anti-Env IgG antibodies targeting the V1V2 region correlated with protection from infection [10–14]. No vaccine-induced virus control was observed in the individuals who became infected, indicating that this vaccine failed to elicit efficient immune responses able to control viremia during the chronic phase of infection. Ideally, an effective vaccine against HIV should provide durable cross-clade humoral immune responses at the portal of entry and arm the cellular immune system with cytotoxic effector memory cells able to contain or eliminate any break-through infection. Therefore, there is urgent need to identify more efficacious vaccine regimens. The use of DNA as vaccine platform is promising due to its simplicity, scalability, and possibility of repeated applications due to the lack of immunity against the vector (for reviews see [15–17]). To date, three DNA vaccines for animal use have been approved: (i) against equine West Nile Virus infection in horses [18,19]; (ii) a therapeutic cancer vaccine against melanoma in dogs [20]; and (iii) a vaccine against infectious hematopoietic necrosis virus (IHNV) in salmon [21–23]. In the HIV vaccine field, few trials have used DNA as the single vaccine modality due to its relatively low immunogenicity in primates, but DNA vaccination was shown to protect macaques from chronic viremia [24–26]. Yet, DNA vaccination methods that optimize immunogen expression and delivery have made great progress in improving immune responses, and it was further shown that DNA vaccines induce long-lasting immunity against HIV/SIV in non-human primates [24,27–29].

2. Regulated HIV *gag* and *env* Expression

The HIV Gag and Env proteins represent key targets for vaccine development. However, from the onset of vaccine development in the mid 80s, HIV DNA as vaccine regimen was found to be poorly immunogenic. Several obstacles needed to be overcome to make DNA a contender in the race of developing an HIV vaccine, including levels of antigen expression and DNA delivery. We now know that the level of antigen expression is critical to induce a potent immune response, but in the past many labs failed to get good expression of HIV Gag and Env, resulting in very poor immunogenicity in animal models. In retrospect, the limited understanding of the rules for efficient gene expression had a serious negative impact resulting in suboptimal levels of antigen production and, subsequently, modest immunogenicity. The reason for this failure is based on the fact, that expression of HIV *gag* and *env* is regulated by the viral Rev protein (for reviews see [30–33] and references therein). Rev is essential for the export of the unspliced and partially spliced mRNAs [34] encoding the structural viral proteins, and mediates production of infectious virions. Rev-minus virus mutants are unable to replicate [35,36], whereas trans-complementation of the Rev-minus HIV with Rev restored expression of *gag* and *env* mRNA as well as virus production, demonstrating the essential role of this regulatory factor. Rev interacts with the *cis*-acting RNA recognition motif termed Rev Responsive Element (RRE) [37,38], a highly structured RNA element embedded within the *env* coding region present only in the unspliced

and partially spliced HIV mRNAs, and promotes their export and expression. The discovery of the HIV Rev mechanism of function opened up new opportunities to understand nucleo-cytoplasmic transport, and posttranscriptional regulation in general, of both viral and cellular mRNAs (reviewed in [30]).

Dissection of the underlying mechanism led to the identification of several regions embedded within the intronic regions in the *gag*, *pol* and *env* coding sequences termed INS (instability sequences) or CRS (*cis*-acting repressive signals) that have a *cis*-acting negative effect on viral mRNA expression [37,39–44] and affect stability, export and expression of this subset of viral mRNAs (see below). Together, the combination of Rev *in trans* and its RNA interaction site RRE *in cis* corrects the defect caused by these negative-acting sequences resulting in efficient Gag/Pol and Env expression and virus production. In conclusion, recognition of this basic regulatory mechanism mediated by the viral Rev protein proved to be the key to achieve efficient expression by HIV *gag* and *env* encoding plasmid DNAs.

3. Method of RNA/Codon Optimization to Circumvent the Poor Expression of HIV *gag/pol* and *env*

The discovery of the role of Rev in the expression of HIV *gag/pol* and *env* provided critical information on how to overcome the poor expression from recombinant vectors containing sub-genomic HIV regions. Yet, this arrangement did not lead to high levels of expression in mouse cells, because the Rev protein does not work efficiently in these cells. This limitation made the study of several recombinant HIV vaccines very challenging or impossible in mice and rabbits, critical models for studying immunogenicity.

The question then arose whether alternative methods could be found to make the HIV *gag* and *env* expression independent of the viral regulatory factor Rev. The initial identification of the negative-acting INS sequences within the coding sequence of *gag* mRNA that were inhibiting expression and affecting the stability of mRNA [40,41], even when placed outside the translated region, suggested they were acting at the level of the mRNA and not during translation at the ribosome. These facts supported the model in which INS/CRS elements provide interaction sites for cellular factors that negatively affect the fate of these transcripts in the nuclear compartment, which consequently affects their fate in the cytoplasm inhibiting translation. This down-regulatory effect can be counteracted by the presence of the HIV posttranscriptional regulatory systems (Rev-RRE). Alternatively, Rev-RRE can be replaced by other RNA export systems such as the retroviral *cis*-acting transport elements CTE or RTE, acting via the cellular NXF1 and RMB15 proteins (reviewed in [30] and references therein). A third method developed by us [40,41] was the removal of the negative-acting RNA instability signals (INS) through RNA/codon optimization.

This alternative method, RNA/codon optimization, which changes the nucleotide sequence without altering the coding potential of the mRNA, was initially achieved by altering the nucleotide composition within eight INS regions in the *gag* mRNA. Changes in 81 of the 1500 nt in the *gag* gene (changing the sequence of 69 of 500 codons in *gag*) without altering the produced protein led to a profound >100-fold increase in Gag protein expression in the absence of Rev/RRE [39–41]. It is now apparent that INS elements interact with many different factors and thus they have diverse nucleotide sequences. Some INS elements contain classical AU-rich elements with the signature motif AUUUA

also found in the 3'UTR of many cytokine and other mRNAs. Such elements are responsible for the posttranscriptional control of many cellular genes. The INS elements exert their function independent of splicing, even when placed 3' of the coding sequence. A proof that INS elements act at the level of mRNA in the nucleus is provided by the fact that INS have no negative effect on expression from recombinant poxvirus vectors (MVA, ALVAC), which produce mRNAs that are confined to the cytoplasm, and therefore “escape” the regulated nuclear export process. In contrast, expression of INS-containing mRNAs from recombinant Adenovirus and Herpes-based vectors is severely affected, because these viruses go to the nucleus and depend on the nuclear export machinery for their expression.

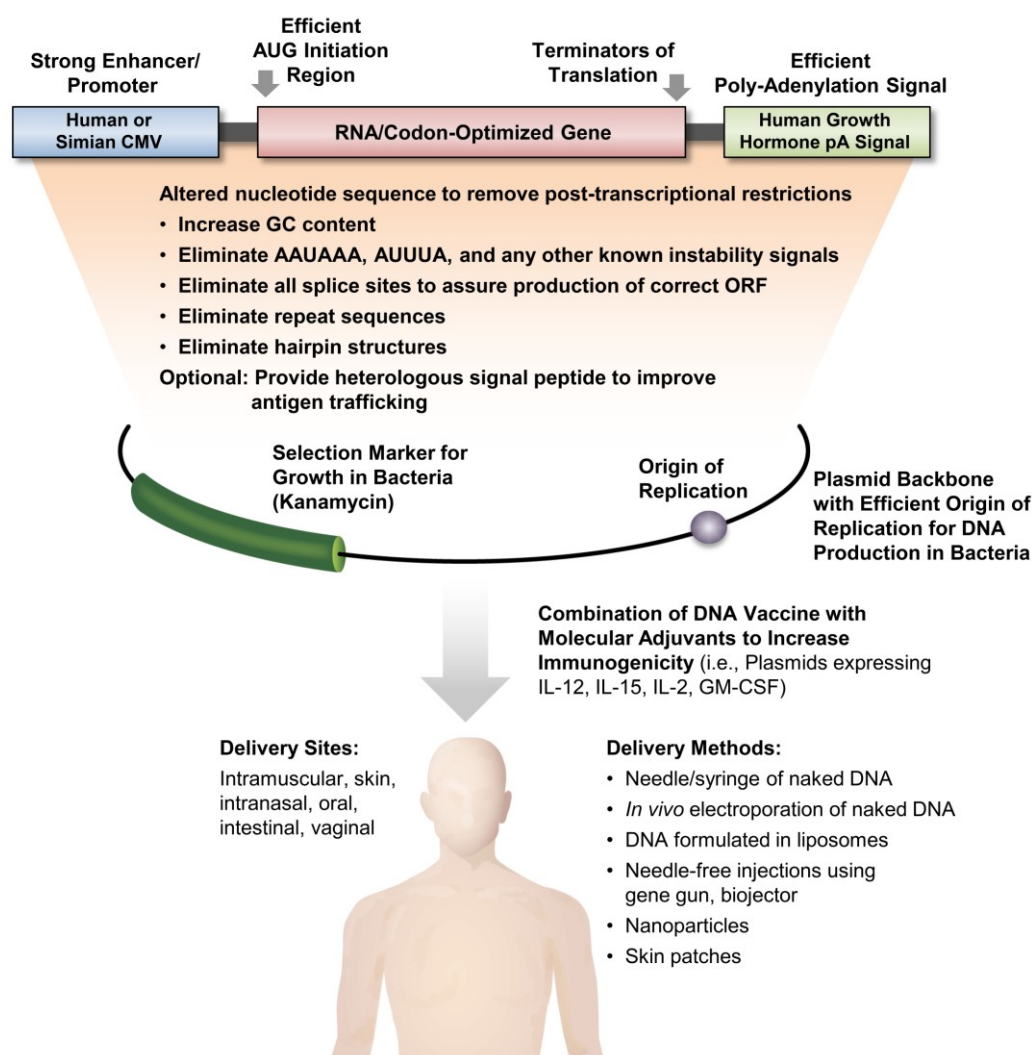
Thus, RNA/codon optimization inactivates the RNA-embedded inhibitory signals (INS) and results in high level Rev-independent production of Gag, Pol and Env (for detailed references see [30]). The discovery of INS/CRS and their effective elimination by RNA optimization have important practical application in the use of such RNA optimized HIV genes in DNA plasmids or recombinant viral vectors currently used in many vaccine studies in monkeys and humans. This general method of RNA/codon optimization is a commonly applied technology for gene transfer studies; it is used successfully for the optimization of numerous viral and cellular mRNAs and results in great gains in mRNA stability, transport and expression. Importantly, most of the candidate AIDS vaccines moving towards the clinic incorporate RNA/codon optimization to achieve efficient antigen expression.

4. Methods to Improve HIV/SIV DNA Vaccine Regimen

Several steps are critical to maximize the efficacy of an HIV DNA vaccine regimen (Figure 1), including optimization of plasmid DNA, optimization of immunogen, DNA delivery method, and the inclusion of molecular adjuvants. (i) To maximize antigen production from plasmid DNA, the RNA/codon optimized genes (see above) are inserted into expression vectors which typically use the human CMV enhancer/promoter and provide a potent initiation of translation signal such as the Kozak sequence (5'-gccgccaccATG(G)-3') or the HIV-1 tat sequence (5'-aagaaATG(G)-3') to initiate translation of the gene of interest and the bovine growth hormone (BGH) polyadenylation signal in a plasmid backbone optimized for replication in bacteria, which may also contain antibiotic genes as selection, typically the kanamycin gene. (ii) A second parameter to improve immunogenicity has also been extensively explored, namely the modification of the natural immunogen. One of the main features of DNA vaccines is versatility and ease of rapid alterations of the expressed immunogen. Although initially the focus was to produce authentic viral proteins as immunogens, it was discovered that modification of the produced immunogen could have advantages as a vaccine. In addition to modifications resulting in increased expression, deletions and fusions of immunogens may lead to improved secretion, rapid degradation or transport to different cellular compartments with the ultimate goal of enhancing immunogenicity. Many such variations have been applied taking advantage of the easy methodology provided by genetic engineering. For HIV/SIV antigens, several methods are employed to improve secretion using signal peptides such as tissue plasminogen activator (tPa) [45,46], or granulocyte-macrophage colony-stimulating factor (GM-CSF) [47]; addition of IgE leader to improve expression [48]; embedding the antigen within LAMP to target the fusion protein to the major histocompatibility complex type II (MHC II) processing compartment [49–51], fusion to MCP-3 to target immunogens to antigen-presenting cells [26,49], and fusion to signals promoting proteasomal

degradation and presentation by the MHC class I molecules such as ubiquitin [52–54] or beta-catenin [26]. (iii) An important milestone in making DNA an attractive vaccine vehicle has been the improvement of *in vivo* delivery. Naked DNA is picked up poorly by primary cells and its expression is minimal. To improve delivery to the nucleus, several methods have been developed including intramuscular DNA delivery by *in vivo* electroporation (IM/EP) ([55–61] reviewed in [62–65]); skin or intradermal electroporation [66–73], skin patches [74], liposome delivery with Vaxfectin® [75,76], DNA formulated in liposomes [77]; gene gun [78] or biojector [79–81]. (iv) Fourth, different strategies to increase the immunogenicity of HIV DNA vaccination in macaques are being pursued, including combination of improved DNA vectors and cytokine DNAs, *i.e.*, IL-12 [82–88], IL-15 [89–91]; IL-2 [89,92–94]; GM-CSF [95–98], chemokines such as RANTES [99] and costimulatory molecules such as CD40L [100–102]. (v) In addition to intramuscular and intradermal routes, DNA can also be delivered via the intranasal, oral, intestinal, and vaginal routes [89,92,93,103].

Figure 1. Optimization of DNA expression and delivery: from bench to bedside. Several steps are necessary to improve the efficiency of DNA as vaccine including RNA/codon optimization of the gene, the use of optimized expression vectors, and combinations of DNA vaccine with molecular adjuvants to increase immunogenicity and using different delivery methods and sites.



We have been focusing on the use IL-12 DNA as molecular adjuvant together with RNA/codon optimized HIV/SIV DNA vaccines in macaque studies [88,104]. Importantly, inclusion of IL-12 resulted in an increase in both vaccine-induced magnitude and breadth of cellular and humoral immunity, even in combination with the efficient electroporation delivery [60,88,104]. In addition, a recent human trial using HIV *gag* DNA showed that inclusion of IL-12 DNA is advantageous, resulting in both increased frequency of responders and level of Gag-specific immunity [105,106], which is in agreement with the data obtained in macaques. Together, the use of IM/EP delivery and inclusion of IL-12 DNA showed a major improvement for HIV DNA vaccine immunogenicity in humans. Previous vaccine trials indicated that the magnitude of immune responses after DNA vaccination using conventional injection methods is low in humans ([107–117]; reviewed in [118]), similar to the conclusions from macaque studies using the same methodology. Clearly, successive studies showed incremental improvements of vaccine-induced immunity. Findings from the macaque model were shown to translate well into clinical trials [105,106,119], validating the ability of this model to identify improved vaccine candidates. As a result of recent studies, there is a strong and renewed interest in DNA vaccines, due to the accumulating evidence of their increased immunogenicity in humans. Importantly, the robust and effective cellular immunity achieved by optimized DNA is also an important consideration for the expanding field of cancer vaccines.

5. HIV/SIV DNA Vaccine Provide Persistent Immunity

A critical feature of any vaccine is the longevity of the induced immune responses. The RV144 vaccine trial in Thailand [9] elicited immune responses that waned rapidly after the vaccination period, suggesting that the transient nature of the elicited immunity could be at least partially responsible for the limited vaccine efficacy. A first study assessing the longevity of the immune responses elicited by EP-delivered DNA vaccines in non-human primates showed persistence of Env humoral responses over one-year of follow-up [120]. We investigated the durability of both cellular and humoral immune responses elicited by the EP delivered DNA vaccine in macaques [28] and reported that SIV DNA vaccination was able to induce persistent immune responses, which were boosted with each subsequent immunization, even after an extended 90-week rest period, indicating long-lasting vaccine-induced immunological memory. We found remarkable durability of Gag and Env immune responses in DNA EP vaccinated macaques for several years [28,29]. Other DNA delivery methods, such as intradermal EP or use of Vaxfectin[®] as a cationic lipid-based formulation, also induced potent immunity with impressive durability detectable for 1–2 years in macaques [76,121,122]. Together, these studies demonstrate another important property of DNA vaccines, which is the remarkable durability of the elicited immune responses.

6. HIV-1 Diversity and DNA Vaccine

HIV sequence diversity and the presence of potential immunodominant “decoy” epitopes are additional hurdles in the development of an effective AIDS vaccine. The plasticity of HIV allows the virus to escape from the immune system generating an enormous number of variants. Another hurdle has been the observation that immunodominant (ID) epitopes present within HIV proteins may impair the induction of more relevant responses [123–134]. These issues need to be taken into consideration

for successful vaccine design. Several approaches are being explored, including strategies that use consensus, center-of-tree or ancestral sequences, combination of multiple strains, mosaic immunogens, which are composed of *in silico* recombined natural sequences, immunogens composed of previously identified epitopes, and chimeric molecules expressing a selection of the most conserved epitopes from different clades of HIV ([47,135–153], reviewed in [154]).

One of our vaccine approaches focuses on the development of immunogens based on strictly conserved elements (CE) of HIV-1 M group [155,156], which induce immune responses to nearly invariable proteome segments, while excluding responses to variable and potentially immunodominant “decoy” epitopes. We developed a prototype CE DNA vaccine and demonstrated that immunization with this conserved element DNA elicited robust cellular and humoral immune responses against CE, which cannot be achieved by full-length immunogen vaccination in mice and macaques [47,153,157]. We demonstrated that priming with CE DNA and boosting with DNA expressing the full-length immunogen is an effective strategy to maximize responses against Gag, providing a novel concept to increase the magnitude and breadth, including epitopes within highly conserved elements, of vaccine-induced immune responses [153,157]. Thus, inclusion of the CE immunogen as part of an HIV-1 vaccine provides a novel and effective vaccine strategy to avoid eliciting responses against potentially immunodominant decoy epitopes, while focusing the responses to critical elements of the virus. The testing of this novel concept in humans will be pursued in a clinical trial.

7. Optimizing Both Arms of the Immune System by DNA & Protein Co-immunization Strategy

Initial efforts for the development of an HIV vaccine showed poor immunogenicity [109], and improved immunogenicity was only found upon using the efficient delivery method of *in vivo* electroporation [105,119,158]. Several vaccines using DNA as a prime in combination with different boosts are also being pursued in clinical trials, including DNA prime-protein boost [114,159], DNA in combination with rMVA [160–165] and DNA prime-rAde boost [8].

As an alternative strategy, we combined the DNA and protein vaccine modalities in a co-immunization regimen. This strategy is based on two observations: (i) DNA vaccination is able to elicit strong cellular immunity, whereas compared to a protein only vaccine, the antibody responses are lower; and (ii) protein vaccination induces strong antibody responses but less robust cellular immunity. Therefore, it was thought that combining these two vaccine regimens into a co-immunization strategy might be ideal to optimally trigger both arms (humoral and cellular) of the immune system. Based on initial observations, co-immunization with DNA delivered by needle and syringe and inactivated SIV virus particles as the protein source induced antibodies that were higher in magnitude and longevity than either component alone [29]. The co-immunization concept was further tested in mice [166,167], rabbits [167] and macaques [24,29,166]. Collectively, it was found that HIV DNA & protein co-immunization was superior in eliciting humoral immune responses to vaccination with either of the two individual components alone, even when DNA was administered by the more efficient EP method. Importantly, HIV DNA & protein co-immunization vaccine regimen did not decrease the magnitude or alter the specificity of the cellular responses [24,29,166]. In addition to inducing the highest systemic binding and cross-neutralizing antibodies to HIV and SIV Env [29,166], this vaccine regimen also

induced the highest Env-specific IgG in saliva [29] and promoted improved dissemination of immune responses to mucosal sites, including rectal fluids [24,29,168].

In conclusion, DNA & protein co-delivery in a simple vaccine regimen combines the strength of each vaccine component, resulting in improved magnitude, extended longevity and increased mucosal dissemination of the induced antibodies in macaques.

8. Efficacy of DNA Vaccine in the Macaque Model

Using DNA as the only vaccine component, we and others previously reported the induction of protective immune responses against SIV or SHIV [24–26,99,169–173], supporting the effectiveness of SIV DNA vaccines to induce potent T cell responses with proliferative capability and multi-functionality, including cytokine secretion and cytotoxicity. Among several parameters, we found that vaccine induced SIV-specific CD4⁺ cytotoxic T cells contributed to control of viremia [24]. Interestingly, such antigen-specific cytotoxic memory CD4⁺ T cells were also found to contribute to virus control in macaques infected with a truly non-pathogenic live-attenuated SIV [174].

Other DNA based vaccination regimens including DNA as prime followed by heterologous boosts, e.g., protein [175–178]; rNYVac [179–181]; rAde [104,182–195]; rHSV [196]; rMVA [87,89,94–96,103,197–204] also reported both immunological benefit and reduction of viremia to different extents. Other prime-boost regimens, e.g., recombinant Rubella vectors [205], also showed promising immunological benefit. Taken together, these studies demonstrate that the DNA vaccine platform, known for its potency as T cell vaccine, also contributes to protection from infection.

Interest in using DNA as a vaccine vehicle for HIV is not limited to preventive vaccine but also for potential application as therapeutic vaccine. Using the SIV/macaque model, we demonstrated virological benefit induced by therapeutic DNA vaccination in SIV macaques under anti-retroviral treatment (ART). DNA vaccination administered by needle and syringe via the IM route or by IM/EP elicited potent cellular responses able to greatly reduce virus load upon release from ART, leading to durable control of viremia over many months [90,206]. Because DNA vaccination can be repeatedly administered without development of immunity to the vector, repeated cycles of therapeutic vaccination resulted in a great benefit with a further reduction in viremia. Recent therapeutic trials using DNA vaccines showed improved immunogenicity ([207–210], for recent review see [211,212]) in agreement with findings of the macaque model. Thus, vaccination with plasmid DNAs has several advantages, including the possibility for repeated administration, and was shown to induce potent, efficacious, and long-lasting immune responses.

9. Perspective and Conclusions

DNA vaccine has great advantages in versatility, scalability, safety and relative simplicity of manufacturing. For HIV, research over the past ~30 years has shown that understanding the basic molecular biology of an immunogen is critical to generating efficient expression vectors, which together with improved DNA delivery, has been able to induce long-lasting cellular and humoral immune responses. Taken together, these findings indicate that DNA-based vaccines are excellent candidates for translation into clinical trials for the development of practical AIDS vaccines.

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Author Contributions

Barbara K. Felber wrote the manuscript. Antonio Valentin, Margherita Rosati, Cristina Bergamaschi and George N. Pavlakis contributed to the writing and editing of this review article.

Conflicts of Interest

Barbara K. Felber and George N. Pavlakis are inventors on US Government-owned patents and patent applications related to DNA vaccines and gene expression optimization.

References

1. Berman, P.W. Development of bivalent rgp120 vaccines to prevent HIV type 1 infection. *AIDS Res. Hum. Retroviruses* **1998**, *14*, S277–S289.
2. Francis, D.P.; Gregory, T.; McElrath, M.J.; Belshe, R.B.; Gorse, G.J.; Migasena, S.; Kitayaporn, D.; Pitisuttitham, P.; Matthews, T.; Schwartz, D.H.; *et al.* Advancing AIDSVAX to phase 3. Safety, immunogenicity, and plans for phase 3. *AIDS Res. Hum. Retroviruses* **1998**, *14*, S325–S331.
3. Pitisuttithum, P.; Gilbert, P.; Gurwith, M.; Heyward, W.; Martin, M.; van Griensven, F.; Hu, D.; Tappero, J.W.; Choopanya, K. 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.* **2006**, *194*, 1661–1671.
4. Flynn, N.M.; Forthal, D.N.; Harro, C.D.; Judson, F.N.; Mayer, K.H.; Para, M.F. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J. Infect. Dis.* **2005**, *191*, 654–665.
5. McElrath, M.J.; de Rosa, S.C.; Moodie, Z.; Dubey, S.; Kierstead, L.; Janes, H.; Defawe, O.D.; Carter, D.K.; Hural, J.; Akondy, R.; *et al.* HIV-1 vaccine-induced immunity in the test-of-concept step study: A case-cohort analysis. *Lancet* **2008**, *372*, 1894–1905.
6. Rolland, M.; Tovanabutra, S.; deCamp, A.C.; Frahm, N.; Gilbert, P.B.; Sanders-Buell, E.; Heath, L.; Magaret, C.A.; Bose, M.; Bradfield, A.; *et al.* Genetic impact of vaccination on breakthrough HIV-1 sequences from the STEP trial. *Nat. Med.* **2011**, *17*, 366–371.
7. Buchbinder, S.P.; Mehrotra, D.V.; Duerr, A.; Fitzgerald, D.W.; Mogg, R.; Li, D.; Gilbert, P.B.; Lama, J.R.; Marmor, M.; del Rio, C.; *et al.* Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): A double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* **2008**, *372*, 1881–1893.
8. Hammer, S.M.; Sobieszczyk, M.E.; Janes, H.; Karuna, S.T.; Mulligan, M.J.; Grove, D.; Koblin, B.A.; Buchbinder, S.P.; Keefer, M.C.; Tomaras, G.D.; *et al.* Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N. Engl. J. Med.* **2013**, *369*, 2083–2092.

9. Rerks-Ngarm, S.; Pitisuttithum, P.; Nitayaphan, S.; Kaewkungwal, J.; Chiu, J.; Paris, R.; Premisri, N.; Namwat, C.; de Souza, M.; Adams, E.; *et al.* Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* **2009**, *361*, 2209–2220.
10. Rolland, M.; Edlefsen, P.T.; Larsen, B.B.; Tovanabutra, S.; Sanders-Buell, E.; Hertz, T.; deCamp, A.C.; Carrico, C.; Menis, S.; Magaret, C.A.; *et al.* Increased HIV-1 vaccine efficacy against viruses with genetic signatures in Env V2. *Nature* **2012**, *490*, 417–420.
11. Haynes, B.F.; Gilbert, P.B.; McElrath, M.J.; Zolla-Pazner, S.; Tomaras, G.D.; Alam, S.M.; Evans, D.T.; Montefiori, D.C.; Karnasuta, C.; Sutthent, R.; *et al.* Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N. Engl. J. Med.* **2012**, *366*, 1275–1286.
12. Gottardo, R.; Bailer, R.T.; Korber, B.T.; Gnanakaran, S.; Phillips, J.; Shen, X.; Tomaras, G.D.; Turk, E.; Imholte, G.; Eckler, L.; *et al.* Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. *PLoS One* **2013**, *8*, e75665.
13. Zolla-Pazner, S.; deCamp, A.C.; Cardozo, T.; Karasavvas, N.; Gottardo, R.; Williams, C.; Morris, D.E.; Tomaras, G.; Rao, M.; Billings, E.; *et al.* Analysis of V2 antibody responses induced in vaccinees in the ALVAC/AIDSVAX HIV-1 vaccine efficacy trial. *PLoS One* **2013**, *8*, e53629.
14. Karasavvas, N.; Billings, E.; Rao, M.; Williams, C.; Zolla-Pazner, S.; Bailer, R.T.; Koup, R.A.; Madnote, S.; Arworn, D.; Shen, X.; *et al.* 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* **2012**, *28*, 1444–1457.
15. Ferraro, B.; Morrow, M.P.; Hutnick, N.A.; Shin, T.H.; Lucke, C.E.; Weiner, D.B. Clinical applications of DNA vaccines: Current progress. *Clin. Infect. Dis.* **2011**, *53*, 296–302.
16. Salenius, K.; Simard, N.; Harland, R.; Ulmer, J.B. The road to licensure of a DNA vaccine. *Curr. Opin. Investig. Drugs* **2007**, *8*, 635–641.
17. Ulmer, J.B.; Wahren, B.; Liu, M.A. DNA vaccines for HIV/AIDS. *Curr. Opin. HIV AIDS* **2006**, *1*, 309–313.
18. Bunning, M.L.; Bowen, R.A.; Cropp, B.; Sullivan, K.; Davis, B.; Komar, N.; Godsey, M.; Baker, D.; Hettler, D.; Holmes, D.; *et al.* Experimental infection of horses with West Nile virus and their potential to infect mosquitoes and serve as amplifying hosts. *Ann. NY Acad. Sci.* **2001**, *951*, 338–339.
19. Davis, B.S.; Chang, G.J.; Cropp, B.; Roehrig, J.T.; Martin, D.A.; Mitchell, C.J.; Bowen, R.; Bunning, M.L. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses *in vitro* a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. *J. Virol.* **2001**, *75*, 4040–4047.
20. Bergman, P.J.; McKnight, J.; Novosad, A.; Charney, S.; Farrelly, J.; Craft, D.; Wulderk, M.; Jeffers, Y.; Sadelain, M.; Hohenhaus, A.E.; *et al.* Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: A phase I trial. *Clin. Cancer Res.* **2003**, *9*, 1284–1290.
21. Traxler, G.S.; Anderson, E.; LaPatra, S.E.; Richard, J.; Shewmaker, B.; Kurath, G. Naked DNA vaccination of Atlantic salmon *Salmo salar* against IHNV. *Dis. Aquat. Organ.* **1999**, *38*, 183–190.
22. Corbeil, S.; Lapatra, S.E.; Anderson, E.D.; Jones, J.; Vincent, B.; Hsu, Y.L.; Kurath, G. Evaluation of the protective immunogenicity of the N, P, M, NV and G proteins of infectious hematopoietic

- necrosis virus in rainbow trout *Oncorhynchus mykiss* using DNA vaccines. *Dis. Aquat. Organ.* **1999**, *39*, 29–36.
23. Garver, K.A.; LaPatra, S.E.; Kurath, G. Efficacy of an infectious hematopoietic necrosis (IHN) virus DNA vaccine in Chinook *Oncorhynchus tshawytscha* and sockeye *O. nerka* salmon. *Dis. Aquat. Organ.* **2005**, *64*, 13–22.
24. Patel, V.; Jalah, R.; Kulkarni, V.; Valentin, A.; Rosati, M.; Alicea, C.; von Gegerfelt, A.; Huang, W.; Guan, Y.; Keele, B.; *et al.* DNA and virus particle vaccination protects against acquisition and confers control of viremia upon heterologous SIV challenge. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 2975–2980.
25. Rosati, M.; Bergamaschi, C.; Valentin, A.; Kulkarni, V.; Jalah, R.; Patel, V.; von Gegerfelt, A.S.; Montefiori, D.C.; Venzon, D.; Khan, A.S.; *et al.* DNA vaccination in rhesus macaques induces potent immune responses and decreases acute and chronic viremia after SIVmac251 challenge. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15831–15836.
26. Rosati, M.; von Gegerfelt, A.; Roth, P.; Alicea, C.; Valentin, A.; Robert-Guroff, M.; Venzon, D.; Montefiori, D.C.; Markham, P.; Felber, B.K.; *et al.* DNA vaccines expressing different forms of simian immunodeficiency virus antigens decrease viremia upon SIVmac251 challenge. *J. Virol.* **2005**, *79*, 8480–8492.
27. Cristillo, A.D.; Galmin, L.; Restrepo, S.; Hudacik, L.; Suschak, J.; Lewis, B.; Draghia-Akli, R.; Aziz, N.; Weiss, D.; Markham, P.; *et al.* HIV-1 Env vaccine comprised of electroporated DNA and protein co-administered with Talabostat. *Biochem. Biophys. Res. Commun.* **2008**, *370*, 22–26.
28. Patel, V.; Valentin, A.; Kulkarni, V.; Rosati, M.; Bergamaschi, C.; Jalah, R.; Alicea, C.; Minang, J.T.; Trivett, M.T.; Ohlen, C.; *et al.* Long-lasting humoral and cellular immune responses and mucosal dissemination after intramuscular DNA immunization. *Vaccine* **2010**, *28*, 4827–4836.
29. Jalah, R.; Kulkarni, V.; Patel, V.; Rosati, M.; Alicea, C.; Bear, J.; Yu, L.; Guan, Y.; Shen, X.; Tomaras, G.D.; *et al.* DNA and protein co-immunization improves the magnitude and longevity of humoral immune responses in rhesus macaques. *PLoS One* **2014**, *9*, e91550.
30. Felber, B.K.; Zolotukhin, A.S.; Pavlakis, G.N. Posttranscriptional control of HIV-1 and other retroviruses and its practical applications. *Adv. Pharmacol.* **2007**, *55*, 161–197.
31. Cochrane, A.W.; McNally, M.T.; Mouland, A.J. The retrovirus RNA trafficking granule: From birth to maturity. *Retrovirology* **2006**, *3*, e18.
32. Chen, C.Y.; Liu, X.; Boris-Lawrie, K.; Sharma, A.; Jeang, K.T. Cellular RNA helicases and HIV-1: Insights from genome-wide, proteomic, and molecular studies. *Virus Res.* **2013**, *171*, 357–365.
33. Yedavalli, V.S.; Jeang, K.T. Rev-ing up post-transcriptional HIV-1 RNA expression. *RNA Biol.* **2011**, *8*, 195–199.
34. Felber, B.K.; Hadzopoulou-Cladaras, M.; Cladaras, C.; Copeland, T.; Pavlakis, G.N. rev Protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1495–1499.
35. Sodroski, J.; Goh, W.C.; Rosen, C.; Dayton, A.; Terwilliger, E.; Haseltine, W. A second post-transcriptional trans-activator gene required for HTLV-III replication. *Nature* **1986**, *321*, 412–417.

36. Feinberg, M.B.; Jarrett, R.F.; Aldovini, A.; Gallo, R.C.; Wong-Staal, F. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. *Cell* **1986**, *46*, 807–817.
37. Hadzopoulou-Cladaras, M.; Felber, B.K.; Cladaras, C.; Athanassopoulos, A.; Tse, A.; Pavlakis, G.N. The *rev* (*trs/art*) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a *cis*-acting sequence in the *env* region. *J. Virol.* **1989**, *63*, 1265–1274.
38. Malim, M.H.; Hauber, J.; Le, S.Y.; Maizel, J.V.; Cullen, B.R. The HIV-1 *rev* *trans*-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature* **1989**, *338*, 254–257.
39. Schneider, R.; Campbell, M.; Nasioulas, G.; Felber, B.K.; Pavlakis, G.N. Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows Rev-independent expression of Gag and Gag/protease and particle formation. *J. Virol.* **1997**, *71*, 4892–4903.
40. Schwartz, S.; Campbell, M.; Nasioulas, G.; Harrison, J.; Felber, B.K.; Pavlakis, G.N. Mutational inactivation of an inhibitory sequence in human immunodeficiency virus type 1 results in Rev-independent gag expression. *J. Virol.* **1992**, *66*, 7176–7182.
41. Schwartz, S.; Felber, B.K.; Pavlakis, G.N. Distinct RNA sequences in the gag region of human immunodeficiency virus type 1 decrease RNA stability and inhibit expression in the absence of Rev protein. *J. Virol.* **1992**, *66*, 150–159.
42. Nasioulas, G.; Zolotukhin, A.S.; Tabernero, C.; Solomin, L.; Cunningham, C.P.; Pavlakis, G.N.; Felber, B.K. Elements distinct from human immunodeficiency virus type 1 splice sites are responsible for the Rev dependence of *env* mRNA. *J. Virol.* **1994**, *68*, 2986–2993.
43. Cochrane, A.W.; Jones, K.S.; Beidas, S.; Dillon, P.J.; Skalka, A.M.; Rosen, C.A. Identification and characterization of intragenic sequences which repress human immunodeficiency virus structural gene expression. *J. Virol.* **1991**, *65*, 5305–5313.
44. Rosen, C.A.; Terwilliger, E.; Dayton, A.; Sodroski, J.G.; Haseltine, W.A. Intragenic *cis*-acting *art* gene-responsive sequences of the human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 2071–2075.
45. Wallace, A.; West, K.; Rothman, A.L.; Ennis, F.A.; Lu, S.; Wang, S. Post-translational intracellular trafficking determines the type of immune response elicited by DNA vaccines expressing Gag antigen of Human Immunodeficiency Virus Type 1 (HIV-1). *Hum. Vaccin Immunother.* **2013**, *9*, 2095–2102.
46. Wang, S.; Farfan-Arribas, D.J.; Shen, S.; Chou, T.H.; Hirsch, A.; He, F.; Lu, S. Relative contributions of codon usage, promoter efficiency and leader sequence to the antigen expression and immunogenicity of HIV-1 Env DNA vaccine. *Vaccine* **2006**, *24*, 4531–4540.
47. Kulkarni, V.; Rosati, M.; Valentin, A.; Ganneru, B.; Singh, A.K.; Yan, J.; Rolland, M.; Alicea, C.; Beach, R.K.; Zhang, G.M.; *et al.* HIV-1 p24gag derived conserved element DNA vaccine increases the breadth of immune response in mice. *PLoS One* **2013**, *8*, e60245.
48. Kumar, S.; Yan, J.; Muthumani, K.; Ramanathan, M.P.; Yoon, H.; Pavlakis, G.N.; Felber, B.K.; Sidhu, M.; Boyer, J.D.; Weiner, D.B. Immunogenicity testing of a novel engineered HIV-1 envelope gp140 DNA vaccine construct. *DNA Cell Biol.* **2006**, *25*, 383–392.
49. Kulkarni, V.; Jalah, R.; Ganneru, B.; Bergamaschi, C.; Alicea, C.; von Gegerfelt, A.; Patel, V.; Zhang, G.M.; Chowdhury, B.; Broderick, K.E.; *et al.* Comparison of immune responses generated

- by optimized DNA vaccination against SIV antigens in mice and macaques. *Vaccine* **2011**, *29*, 6742–6754.
50. Valentin, A.; Chikhlikar, P.; Patel, V.; Rosati, M.; Maciel, M.; Chang, K.H.; Silvera, P.; Felber, B.K.; Pavlakis, G.N.; August, J.T.; *et al.* Comparison of DNA vaccines producing HIV-1 Gag and LAMP/Gag chimera in rhesus macaques reveals antigen-specific T-cell responses with distinct phenotypes. *Vaccine* **2009**, *27*, 4840–4849.
 51. Chikhlikar, P.; de Arruda, L.B.; Maciel, M.; Silvera, P.; Lewis, M.G.; August, J.T.; Marques, E.T. DNA encoding an HIV-1 Gag/human lysosome-associated membrane protein-1 chimera elicits a broad cellular and humoral immune response in rhesus macaques. *PLoS One* **2006**, *1*, e135.
 52. Benlahrech, A.; Meiser, A.; Herath, S.; Papagatsias, T.; Athanasopoulos, T.; Li, F.; Self, S.; Bachy, V.; Hervouet, C.; Logan, K.; *et al.* Fragmentation of SIV-gag vaccine induces broader T cell responses. *PLoS One* **2012**, *7*, e48038.
 53. Stack, J.H.; Whitney, M.; Rodems, S.M.; Pollok, B.A. A ubiquitin-based tagging system for controlled modulation of protein stability. *Nat. Biotechnol.* **2000**, *18*, 1298–1302.
 54. Tobery, T.W.; Siliciano, R.F. Targeting of HIV-1 antigens for rapid intracellular degradation enhances cytotoxic T lymphocyte (CTL) recognition and the induction of *de novo* CTL responses *in vivo* after immunization. *J. Exp. Med.* **1997**, *185*, 909–920.
 55. Mathiesen, I. Electroporation of skeletal muscle enhances gene transfer *in vivo*. *Gene Ther.* **1999**, *6*, 508–514.
 56. Heller, L.; Pottinger, C.; Jaroszeski, M.J.; Gilbert, R.; Heller, R. *In vivo* electroporation of plasmids encoding GM-CSF or interleukin-2 into existing B16 melanomas combined with electrochemotherapy induces long-term antitumour immunity. *Melanoma Res.* **2000**, *10*, 577–583.
 57. Widera, G.; Austin, M.; Rabussay, D.; Goldbeck, C.; Barnett, S.W.; Chen, M.; Leung, L.; Otten, G.R.; Thudium, K.; Selby, M.J.; *et al.* Increased DNA vaccine delivery and immunogenicity by electroporation *in vivo*. *J. Immunol.* **2000**, *164*, 4635–4640.
 58. Selby, M.; Goldbeck, C.; Pertile, T.; Walsh, R.; Ulmer, J. Enhancement of DNA vaccine potency by electroporation *in vivo*. *J. Biotechnol.* **2000**, *83*, 147–152.
 59. Otten, G.; Schaefer, M.; Doe, B.; Liu, H.; Srivastava, I.; Megede, J.Z.; O'Hagan, D.; Donnelly, J.; Widera, G.; Rabussay, D.; *et al.* Enhancement of DNA vaccine potency in rhesus macaques by electroporation. *Vaccine* **2004**, *22*, 2489–2493.
 60. Hirao, L.A.; Wu, L.; Khan, A.S.; Hokey, D.A.; Yan, J.; Dai, A.; Betts, M.R.; Draghia-Akli, R.; Weiner, D.B. Combined effects of IL-12 and electroporation enhances the potency of DNA vaccination in macaques. *Vaccine* **2008**, *26*, 3112–3120.
 61. Rosati, M.; Valentin, A.; Jalah, R.; Patel, V.; von Gegerfelt, A.; Bergamaschi, C.; Alicea, C.; Weiss, D.; Treece, J.; Pal, R.; *et al.* Increased immune responses in rhesus macaques by DNA vaccination combined with electroporation. *Vaccine* **2008**, *26*, 5223–5229.
 62. Sardesai, N.Y.; Weiner, D.B. Electroporation delivery of DNA vaccines: Prospects for success. *Curr. Opin. Immunol.* **2011**, *23*, 421–429.
 63. Hutnick, N.A.; Myles, D.J.; Bian, C.B.; Muthumani, K.; Weiner, D.B. Selected approaches for increasing HIV DNA vaccine immunogenicity *in vivo*. *Curr. Opin. Virol.* **2012**, *1*, 233–240.
 64. Bodles-Brakhop, A.M.; Heller, R.; Draghia-Akli, R. Electroporation for the delivery of DNA-based vaccines and immunotherapeutics: Current clinical developments. *Mol. Ther.* **2009**, *17*, 585–592.

65. Flingai, S.; Czerwonko, M.; Goodman, J.; Kudchodkar, S.B.; Muthumani, K.; Weiner, D.B. Synthetic DNA vaccines: Improved vaccine potency by electroporation and co-delivered genetic adjuvants. *Front. Immunol.* **2013**, *4*, doi:10.3389/fimmu.2013.00354.
66. Hallengard, D.; Brave, A.; Isaguliant, M.; Blomberg, P.; Enger, J.; Stout, R.; King, A.; Wahren, B. A combination of intradermal jet-injection and electroporation overcomes *in vivo* dose restriction of DNA vaccines. *Genet. Vaccines Ther.* **2012**, *10*, doi:10.1186/1479-0556-10-5.
67. Roos, A.K.; Eriksson, F.; Timmons, J.A.; Gerhardt, J.; Nyman, U.; Gudmundsdotter, L.; Brave, A.; Wahren, B.; Pisa, P. Skin electroporation: Effects on transgene expression, DNA persistence and local tissue environment. *PLoS One* **2009**, *4*, e7226.
68. Hooper, J.W.; Golden, J.W.; Ferro, A.M.; King, A.D. Smallpox DNA vaccine delivered by novel skin electroporation device protects mice against intranasal poxvirus challenge. *Vaccine* **2007**, *25*, 1814–1823.
69. Brave, A.; Gudmundsdotter, L.; Sandstrom, E.; Haller, B.K.; Hallengard, D.; Maltais, A.K.; King, A.D.; Stout, R.R.; Blomberg, P.; Hoglund, U.; *et al.* Biodistribution, persistence and lack of integration of a multigene HIV vaccine delivered by needle-free intradermal injection and electroporation. *Vaccine* **2010**, *28*, 8203–8209.
70. Hutnick, N.A.; Myles, D.J.; Ferraro, B.; Lucke, C.; Lin, F.; Yan, J.; Broderick, K.E.; Khan, A.S.; Sardesai, N.Y.; Weiner, D.B. Intradermal DNA vaccination enhanced by low-current electroporation improves antigen expression and induces robust cellular and humoral immune responses. *Hum. Gene Ther.* **2012**, *23*, 943–950.
71. Lin, F.; Shen, X.; Kichaev, G.; Mendoza, J.M.; Yang, M.; Armendi, P.; Yan, J.; Kobinger, G.P.; Bello, A.; Khan, A.S.; *et al.* Optimization of electroporation-enhanced intradermal delivery of DNA vaccine using a minimally invasive surface device. *Hum. Gene Ther. Methods* **2012**, *23*, 157–168.
72. Kichaev, G.; Mendoza, J.M.; Amante, D.; Smith, T.R.; McCoy, J.R.; Sardesai, N.Y.; Broderick, K.E. Electroporation mediated DNA vaccination directly to a mucosal surface results in improved immune responses. *Hum. Vaccin Immunother.* **2013**, *9*, 2041–2048.
73. Heller, R.; Cruz, Y.; Heller, L.C.; Gilbert, R.A.; Jaroszeski, M.J. Electrically mediated delivery of plasmid DNA to the skin, using a multielectrode array. *Hum. Gene Ther.* **2010**, *21*, 357–362.
74. Lori, F. DermaVir: A plasmid DNA-based nanomedicine therapeutic vaccine for the treatment of HIV/AIDS. *Expert Rev. Vaccines* **2011**, *10*, 1371–1384.
75. Sullivan, S.M.; Doukas, J.; Hartikka, J.; Smith, L.; Rolland, A. Vaxfectin: A versatile adjuvant for plasmid DNA- and protein-based vaccines. *Expert Opin. Drug Deliv.* **2010**, *7*, 1433–1446.
76. Kulkarni, V.; Rosati, M.; Valentin, A.; Jalah, R.; Alicea, C.; Yu, L.; Guan, Y.; Shen, X.; Tomaras, G.D.; LaBranche, C.; *et al.* Vaccination with Vaxfectin[®] adjuvanted SIV DNA induces long-lasting humoral immune responses able to reduce SIVmac251 Viremia. *Hum. Vaccin Immunother.* **2013**, *9*, 2069–2080.
77. Templeton, N.S.; Lasic, D.D.; Frederik, P.M.; Strey, H.H.; Roberts, D.D.; Pavlakis, G.N. Improved DNA: Liposome complexes for increased systemic delivery and gene expression. *Nat. Biotechnol.* **1997**, *15*, 647–652.
78. Fuller, D.H.; Simpson, L.; Cole, K.S.; Clements, J.E.; Panicali, D.L.; Montelaro, R.C.; Murphey-Corb, M.; Haynes, J.R. Gene gun-based nucleic acid immunization alone or in combination

- with recombinant vaccinia vectors suppresses virus burden in rhesus macaques challenged with a heterologous SIV. *Immunol. Cell Biol.* **1997**, *75*, 389–396.
79. Aguiar, J.C.; Hedstrom, R.C.; Rogers, W.O.; Charoenvit, Y.; Sacci, J.B., Jr.; Lanar, D.E.; Majam, V.F.; Stout, R.R.; Hoffman, S.L. Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. *Vaccine* **2001**, *20*, 275–280.
80. Jackson, L.A.; Austin, G.; Chen, R.T.; Stout, R.; DeStefano, F.; Gorse, G.J.; Newman, F.K.; Yu, O.; Weniger, B.G. Safety and immunogenicity of varying dosages of trivalent inactivated influenza vaccine administered by needle-free jet injectors. *Vaccine* **2001**, *19*, 4703–4709.
81. Williams, J.; Fox-Leyva, L.; Christensen, C.; Fisher, D.; Schlichting, E.; Snowball, M.; Negus, S.; Mayers, J.; Koller, R.; Stout, R. Hepatitis A vaccine administration: Comparison between jet-injector and needle injection. *Vaccine* **2000**, *18*, 1939–1943.
82. Boyer, J.D.; Robinson, T.M.; Kutzler, M.A.; Parkinson, R.; Calarota, S.A.; Sidhu, M.K.; Muthumani, K.; Lewis, M.; Pavlakis, G.; Felber, B.; *et al.* SIV DNA vaccine co-administered with IL-12 expression plasmid enhances CD8 SIV cellular immune responses in *Cynomolgus macaques*. *J. Med. Primatol.* **2005**, *34*, 262–270.
83. Chong, S.Y.; Egan, M.A.; Kutzler, M.A.; Megati, S.; Masood, A.; Roopchand, V.; Garcia-Hand, D.; Montefiori, D.C.; Quiroz, J.; Rosati, M.; *et al.* Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. *Vaccine* **2007**, *25*, 4967–4982.
84. Schadeck, E.B.; Sidhu, M.; Egan, M.A.; Chong, S.Y.; Piacente, P.; Masood, A.; Garcia-Hand, D.; Cappello, S.; Roopchand, V.; Megati, S.; *et al.* A dose sparing effect by plasmid encoded IL-12 adjuvant on a SIVgag-plasmid DNA vaccine in rhesus macaques. *Vaccine* **2006**, *24*, 4677–4687.
85. Robinson, T.M.; Sidhu, M.K.; Pavlakis, G.N.; Felber, B.K.; Silvera, P.; Lewis, M.G.; Eldridge, J.; Weiner, D.B.; Boyer, J.D. Macaques co-immunized with SIVgag/pol-HIVenv and IL-12 plasmid have increased cellular responses. *J. Med. Primatol.* **2007**, *36*, 276–284.
86. Halwani, R.; Boyer, J.D.; Yassine-Diab, B.; Haddad, E.K.; Robinson, T.M.; Kumar, S.; Parkinson, R.; Wu, L.; Sidhu, M.K.; Phillipson-Weiner, R.; *et al.* Therapeutic vaccination with simian immunodeficiency virus (SIV)-DNA+IL-12 or IL-15 induces distinct CD8 memory subsets in SIV-infected macaques. *J. Immunol.* **2008**, *180*, 7969–7979.
87. Manrique, M.; Micewicz, E.; Kozlowski, P.A.; Wang, S.W.; Aurora, D.; Wilson, R.L.; Ghebremichael, M.; Mazzara, G.; Montefiori, D.; Carville, A.; *et al.* DNA-MVA vaccine protection after X4 SHIV challenge in macaques correlates with day-of-challenge antiviral CD4⁺ cell-mediated immunity levels and postchallenge preservation of CD4⁺ T cell memory. *AIDS Res. Hum. Retrovir.* **2008**, *24*, 505–519.
88. Jalah, R.; Patel, V.; Kulkarni, V.; Rosati, M.; Alicea, C.; Ganneru, B.; von Gegerfelt, A.; Huang, W.; Guan, Y.; Broderick, K.E.; *et al.* IL-12 DNA as molecular vaccine adjuvant increases the cytotoxic T cell responses and breadth of humoral immune responses in SIV DNA vaccinated macaques. *Hum. Vaccin Immunother.* **2012**, *8*, 1620–1629.
89. Manrique, M.; Kozlowski, P.A.; Wang, S.W.; Wilson, R.L.; Micewicz, E.; Montefiori, D.C.; Mansfield, K.G.; Carville, A.; Aldovini, A. Nasal DNA-MVA SIV vaccination provides more

- significant protection from progression to AIDS than a similar intramuscular vaccination. *Mucosal Immunol.* **2009**, *2*, 536–550.
90. Valentin, A.; von Gegerfelt, A.; Rosati, M.; Miteloudis, G.; Alicea, C.; Bergamaschi, C.; Jalah, R.; Patel, V.; Khan, A.S.; Draghia-Akli, R.; *et al.* Repeated DNA therapeutic vaccination of chronically SIV-infected macaques provides additional virological benefit. *Vaccine* **2010**, *28*, 1962–1974.
91. Calarota, S.A.; Dai, A.; Trocio, J.N.; Weiner, D.B.; Lori, F.; Lisziewicz, J. IL-15 as memory T-cell adjuvant for topical HIV-1 DermaVir vaccine. *Vaccine* **2008**, *26*, 5188–5195.
92. Manrique, M.; Kozlowski, P.A.; Cobo-Molinos, A.; Wang, S.W.; Wilson, R.L.; Mdel, P.M.-V.; Montefiori, D.C.; Carville, A.; Aldovini, A. Resistance to infection, early and persistent suppression of simian immunodeficiency virus SIVmac251 viremia, and significant reduction of tissue viral burden after mucosal vaccination in female rhesus macaques. *J. Virol.* **2014**, *88*, 212–224.
93. Manrique, M.; Kozlowski, P.A.; Cobo-Molinos, A.; Wang, S.W.; Wilson, R.L.; Montefiori, D.C.; Carville, A.; Aldovini, A. Immunogenicity of a vaccine regimen composed of simian immunodeficiency virus DNA, rMVA, and viral particles administered to female rhesus macaques via four different mucosal routes. *J. Virol.* **2013**, *87*, 4738–4750.
94. Manrique, M.; Kozlowski, P.A.; Cobo-Molinos, A.; Wang, S.W.; Wilson, R.L.; Montefiori, D.C.; Mansfield, K.G.; Carville, A.; Aldovini, A. Long-term control of simian immunodeficiency virus mac251 viremia to undetectable levels in half of infected female rhesus macaques nasally vaccinated with simian immunodeficiency virus DNA/recombinant modified vaccinia virus Ankara. *J. Immunol.* **2011**, *186*, 3581–3593.
95. Lai, L.; Kwa, S.; Kozlowski, P.A.; Montefiori, D.C.; Ferrari, G.; Johnson, W.E.; Hirsch, V.; Villinger, F.; Chennareddi, L.; Earl, P.L.; *et al.* Prevention of infection by a granulocyte-macrophage colony-stimulating factor co-expressing DNA/modified vaccinia ankara simian immunodeficiency virus vaccine. *J. Infect. Dis.* **2011**, *204*, 164–173.
96. Lai, L.; Vodros, D.; Kozlowski, P.A.; Montefiori, D.C.; Wilson, R.L.; Akerstrom, V.L.; Chennareddi, L.; Yu, T.; Kannanganat, S.; Ofielu, L.; *et al.* GM-CSF DNA: An adjuvant for higher avidity IgG, rectal IgA, and increased protection against the acute phase of a SHIV-89.6P challenge by a DNA/MVA immunodeficiency virus vaccine. *Virology* **2007**, *369*, 153–167.
97. O'Neill, E.; Bostik, V.; Montefiori, D.C.; Kraiselburd, E.; Villinger, F. IL-12/GM-CSF coadministration in an SIV DNA prime/protein boost protocol enhances Gag-specific T cells but not virus-specific neutralizing antibodies in rhesus macaques. *AIDS Res. Hum. Retrovir.* **2003**, *19*, 883–890.
98. Hellerstein, M.; Xu, Y.; Marino, T.; Lu, S.; Yi, H.; Wright, E.R.; Robinson, H.L. Co-expression of HIV-1 virus-like particles and granulocyte-macrophage colony stimulating factor by GEO-D03 DNA vaccine. *Hum. Vaccin. Immunother.* **2012**, *8*, 1654–1658.
99. Belisle, S.E.; Yin, J.; Shedlock, D.J.; Dai, A.; Yan, J.; Hirao, L.; Kutzler, M.A.; Lewis, M.G.; Andersen, H.; Lank, S.M.; *et al.* Long-term programming of antigen-specific immunity from gene expression signatures in the PBMC of rhesus macaques immunized with an SIV DNA vaccine. *PLoS One* **2011**, *6*, e19681.

100. Ihata, A.; Watabe, S.; Sasaki, S.; Shirai, A.; Fukushima, J.; Hamajima, K.; Inoue, J.; Okuda, K. Immunomodulatory effect of a plasmid expressing CD40 ligand on DNA vaccination against human immunodeficiency virus type-1. *Immunology* **1999**, *98*, 436–442.
101. Liu, J.; Yu, Q.; Stone, G.W.; Yue, F.Y.; Ngai, N.; Jones, R.B.; Kornbluth, R.S.; Ostrowski, M.A. CD40L expressed from the canarypox vector, ALVAC, can boost immunogenicity of HIV-1 canarypox vaccine in mice and enhance the *in vitro* expansion of viral specific CD8⁺ T cell memory responses from HIV-1-infected and HIV-1-uninfected individuals. *Vaccine* **2008**, *26*, 4062–4072.
102. Franco, D.; Liu, W.; Gardiner, D.F.; Hahn, B.H.; Ho, D.D. CD40L-containing virus-like particle as a candidate HIV-1 vaccine targeting dendritic cells. *J. Acquir. Immune Defic. Syndr.* **2011**, *56*, 393–400.
103. Wang, S.W.; Bertley, F.M.; Kozlowski, P.A.; Herrmann, L.; Manson, K.; Mazzara, G.; Piatak, M.; Johnson, R.P.; Carville, A.; Mansfield, K.; *et al.* An SHIV DNA/MVA rectal vaccination in macaques provides systemic and mucosal virus-specific responses and protection against AIDS. *AIDS Res. Hum. Retroviruses* **2004**, *20*, 846–859.
104. Winstone, N.; Wilson, A.J.; Morrow, G.; Boggiano, C.; Chiuchiolo, M.J.; Lopez, M.; Kemelman, M.; Ginsberg, A.A.; Mullen, K.; Coleman, J.W.; *et al.* Enhanced control of pathogenic SIVmac239 replication in macaques immunized with a plasmid IL12 and a DNA prime, viral vector boost vaccine regimen. *J. Virol.* **2011**, *85*, 9578–9587.
105. Kalams, S.A.; Parker, S.; Jin, X.; Elizaga, M.; Metch, B.; Wang, M.; Hural, J.; Lubeck, M.; Eldridge, J.; Cardinali, M.; *et al.* Safety and immunogenicity of an HIV-1 gag DNA vaccine with or without IL-12 and/or IL-15 plasmid cytokine adjuvant in healthy, HIV-1 uninfected adults. *PLoS One* **2012**, *7*, e29231.
106. Kalams, S.A.; Parker, S.D.; Elizaga, M.; Metch, B.; Edupuganti, S.; Hural, J.; de Rosa, S.; Carter, D.K.; Rybczyk, K.; Frank, I.; *et al.* Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. *J. Infect. Dis.* **2013**, *208*, 818–829.
107. Tavel, J.A.; Martin, J.E.; Kelly, G.G.; Enama, M.E.; Shen, J.M.; Gomez, P.L.; Andrews, C.A.; Koup, R.A.; Bailer, R.T.; Stein, J.A.; *et al.* Safety and immunogenicity of a Gag-Pol candidate HIV-1 DNA vaccine administered by a needle-free device in HIV-1-seronegative subjects. *J. Acquir. Immune Defic. Syndr.* **2007**, *44*, 601–605.
108. Wang, Z.; Troilo, P.J.; Wang, X.; Griffiths, T.G.; Pacchione, S.J.; Barnum, A.B.; Harper, L.B.; Pauley, C.J.; Niu, Z.; Denisova, L.; *et al.* Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. *Gene Ther.* **2004**, *11*, 711–721.
109. Graham, B.S.; Koup, R.A.; Roederer, M.; Bailer, R.T.; Enama, M.E.; Moodie, Z.; Martin, J.E.; McCluskey, M.M.; Chakrabarti, B.K.; Lamoreaux, L.; *et al.* Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 DNA candidate vaccine. *J. Infect. Dis.* **2006**, *194*, 1650–1660.
110. Mwau, M.; Cebere, I.; Sutton, J.; Chikoti, P.; Winstone, N.; Wee, E.G.; Beattie, T.; Chen, Y.H.; Dorrell, L.; McShane, H.; *et al.* A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: Stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. *J. Gen. Virol.* **2004**, *85*, 911–919.

111. Bansal, A.; Jackson, B.; West, K.; Wang, S.; Lu, S.; Kennedy, J.S.; Goepfert, P.A. Multifunctional T-cell characteristics induced by a polyvalent DNA prime/protein boost human immunodeficiency virus type 1 vaccine regimen given to healthy adults are dependent on the route and dose of administration. *J. Virol.* **2008**, *82*, 6458–6469.
112. Jaoko, W.; Nakwagala, F.N.; Anzala, O.; Manyonyi, G.O.; Birungi, J.; Nanvubya, A.; Bashir, F.; Bhatt, K.; Ogutu, H.; Wakasiaka, S.; *et al.* Safety and immunogenicity of recombinant low-dosage HIV-1 A vaccine candidates vectored by plasmid pTHr DNA or modified vaccinia virus Ankara (MVA) in humans in East Africa. *Vaccine* **2008**, *26*, 2788–2795.
113. Wilson, C.C.; Newman, M.J.; Livingston, B.D.; MaWhinney, S.; Forster, J.E.; Scott, J.; Schooley, R.T.; Benson, C.A. Clinical phase 1 testing of the safety and immunogenicity of an epitope-based DNA vaccine in human immunodeficiency virus type 1-infected subjects receiving highly active antiretroviral therapy. *Clin. Vaccine Immunol.* **2008**, *15*, 986–994.
114. Wang, S.; Kennedy, J.S.; West, K.; Montefiori, D.C.; Coley, S.; Lawrence, J.; Shen, S.; Green, S.; Rothman, A.L.; Ennis, F.A.; *et al.* Cross-subtype antibody and cellular immune responses induced by a polyvalent DNA prime-protein boost HIV-1 vaccine in healthy human volunteers. *Vaccine* **2008**, *26*, 1098–1110.
115. Gorse, G.J.; Baden, L.R.; Wecker, M.; Newman, M.J.; Ferrari, G.; Weinhold, K.J.; Livingston, B.D.; Villafana, T.L.; Li, H.; Noonan, E.; *et al.* Safety and immunogenicity of cytotoxic T-lymphocyte poly-epitope, DNA plasmid (EP HIV-1090) vaccine in healthy, human immunodeficiency virus type 1 (HIV-1)-uninfected adults. *Vaccine* **2008**, *26*, 215–223.
116. Eller, M.A.; Eller, L.A.; Opollo, M.S.; Ouma, B.J.; Oballah, P.O.; Galley, L.; Karnasuta, C.; Kim, S.R.; Robb, M.L.; Michael, N.L.; *et al.* Induction of HIV-specific functional immune responses by a multiclade HIV-1 DNA vaccine candidate in healthy Ugandans. *Vaccine* **2007**, *25*, 7737–7742.
117. Catanzaro, A.T.; Roederer, M.; Koup, R.A.; Bailer, R.T.; Enama, M.E.; Nason, M.C.; Martin, J.E.; Rucker, S.; Andrews, C.A.; Gomez, P.L.; *et al.* Phase I clinical evaluation of a six-plasmid multiclade HIV-1 DNA candidate vaccine. *Vaccine* **2007**, *25*, 4085–4092.
118. Kutzler, M.A.; Weiner, D.B. DNA vaccines: Ready for prime time? *Nat. Rev. Genet.* **2008**, *9*, 776–788.
119. Vasan, S.; Hurley, A.; Schlesinger, S.J.; Hannaman, D.; Gardiner, D.F.; Dugin, D.P.; Boente-Carrera, M.; Vittorino, R.; Caskey, M.; Andersen, J.; *et al.* *In vivo* electroporation enhances the immunogenicity of an HIV-1 DNA vaccine candidate in healthy volunteers. *PLoS One* **2011**, *6*, e19252.
120. Cristillo, A.D.; Weiss, D.; Hudacik, L.; Restrepo, S.; Galmin, L.; Suschak, J.; Draghia-Akli, R.; Markham, P.; Pal, R. Persistent antibody and T cell responses induced by HIV-1 DNA vaccine delivered by electroporation. *Biochem. Biophys. Res. Commun.* **2008**, *366*, 29–35.
121. Kulkarni, V.; Rosati, M.; Bear, J.; Pilkington, G.R.; Jalah, R.; Bergamaschi, C.; Singh, A.K.; Alicea, C.; Chowdhury, B.; Zhang, G.-M.; *et al.* Comparison of intradermal and intramuscular delivery of SIV env DNA by *in vivo* electroporation in macaques. *Hum. Vaccin Immunother.* **2013**, *9*, 2081–2094.

122. Kulkarni, V.; Rosati, M.; Jalah, R.; Ganneru, B.; Alicea, C.; Yu, L.; Guan, Y.; LaBranche, C.; Montefiori, D.C.; King, A.D.; *et al.* DNA vaccination by intradermal electroporation induces long-lasting immune responses in Rhesus macaques. *J. Med. Primatol.*, in press.
123. Lichterfeld, M.; Yu, X.G.; le Gall, S.; Altfeld, M. Immunodominance of HIV-1-specific CD8⁺ T-cell responses in acute HIV-1 infection: At the crossroads of viral and host genetics. *Trends Immunol.* **2005**, *26*, 166–171.
124. Friedrich, T.C.; Valentine, L.E.; Yant, L.J.; Rakasz, E.G.; Piaskowski, S.M.; Furlott, J.R.; Weisgrau, K.L.; Burwitz, B.; May, G.E.; Leon, E.J.; *et al.* Subdominant CD8⁺ T-cell responses are involved in durable control of AIDS virus replication. *J. Virol.* **2007**, *81*, 3465–3476.
125. Liu, Y.; McNevin, J.; Rolland, M.; Zhao, H.; Deng, W.; Maenza, J.; Stevens, C.E.; Collier, A.C.; McElrath, M.J.; Mullins, J.I. Conserved HIV-1 epitopes continuously elicit subdominant cytotoxic T-lymphocyte responses. *J. Infect. Dis.* **2009**, *200*, 1825–1833.
126. Liu, J.; Ewald, B.A.; Lynch, D.M.; Nanda, A.; Sumida, S.M.; Barouch, D.H. Modulation of DNA vaccine-elicited CD8⁺ T-lymphocyte epitope immunodominance hierarchies. *J. Virol.* **2006**, *80*, 11991–11997.
127. Frahm, N.; Kiepiela, P.; Adams, S.; Linde, C.H.; Hewitt, H.S.; Sango, K.; Feeney, M.E.; Addo, M.M.; Lichterfeld, M.; Lahaie, M.P.; *et al.* Control of human immunodeficiency virus replication by cytotoxic T lymphocytes targeting subdominant epitopes. *Nat. Immunol.* **2006**, *7*, 173–178.
128. Bockl, K.; Wild, J.; Bredl, S.; Kindsmuller, K.; Kostler, J.; Wagner, R. Altering an artificial gagpolnef polyprotein and mode of env co-administration affects the immunogenicity of a clade C HIV DNA vaccine. *PLoS One* **2012**, *7*, e34723.
129. Iversen, A.K.; Stewart-Jones, G.; Learn, G.H.; Christie, N.; Sylvester-Hviid, C.; Armitage, A.E.; Kaul, R.; Beattie, T.; Lee, J.K.; Li, Y.; *et al.* Conflicting selective forces affect T cell receptor contacts in an immunodominant human immunodeficiency virus epitope. *Nat. Immunol.* **2006**, *7*, 179–189.
130. Schneidewind, A.; Brumme, Z.L.; Brumme, C.J.; Power, K.A.; Reyor, L.L.; O’Sullivan, K.; Gladden, A.; Hempel, U.; Kuntzen, T.; Wang, Y.E.; *et al.* Transmission and long-term stability of compensated CD8 escape mutations. *J. Virol.* **2009**, *83*, 3993–3997.
131. Altfeld, M.; Kalife, E.T.; Qi, Y.; Streeck, H.; Lichterfeld, M.; Johnston, M.N.; Burgett, N.; Swartz, M.E.; Yang, A.; Alter, G.; *et al.* HLA alleles associated with delayed progression to AIDS contribute strongly to the initial CD8⁺ T cell response against HIV-1. *PLoS Med.* **2006**, *3*, e403.
132. Friedrich, D.; Jalbert, E.; Dinges, W.L.; Sidney, J.; Sette, A.; Huang, Y.; McElrath, M.J.; Horton, H. Vaccine-induced HIV-specific CD8⁺ T cells utilize preferential HLA alleles and target-specific regions of HIV-1. *J. Acquir. Immune Defic. Syndr.* **2011**, *58*, 248–252.
133. Maurer, K.; Harrer, E.G.; Goldwisch, A.; Eismann, K.; Bergmann, S.; Schmitt-Haendle, M.; Spriewald, B.; Mueller, S.M.; Harrer, T. Role of cytotoxic T-lymphocyte-mediated immune selection in a dominant human leukocyte antigen-B8-restricted cytotoxic T-lymphocyte epitope in Nef. *J. Acquir. Immune Defic. Syndr.* **2008**, *48*, 133–141.
134. Li, F.; Finnefrock, A.C.; Dubey, S.A.; Korber, B.T.; Szinger, J.; Cole, S.; McElrath, M.J.; Shiver, J.W.; Casimiro, D.R.; Corey, L.; *et al.* Mapping HIV-1 vaccine induced T-cell responses:

- Bias towards less-conserved regions and potential impact on vaccine efficacy in the step study. *PLoS One* **2011**, *6*, e20479.
135. Nickle, D.C.; Rolland, M.; Jensen, M.A.; Pond, S.L.; Deng, W.; Seligman, M.; Heckerman, D.; Mullins, J.I.; Jojic, N. Coping with viral diversity in HIV vaccine design. *PLoS Comput. Biol.* **2007**, *3*, e75.
 136. Nickle, D.C.; Jojic, N.; Heckerman, D.; Jojic, V.; Kirovski, D.; Rolland, M.; Pond, S.K.; Mullins, J.I. Comparison of immunogen designs that optimize peptide coverage: Reply to Fischer *et al.* *PLoS Comput. Biol.* **2008**, *4*, e25.
 137. Barouch, D.H.; O'Brien, K.L.; Simmons, N.L.; King, S.L.; Abbink, P.; Maxfield, L.F.; Sun, Y.H.; la Porte, A.; Riggs, A.M.; Lynch, D.M.; *et al.* Mosaic HIV-1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. *Nat. Med.* **2010**, *16*, 319–323.
 138. Santra, S.; Liao, H.X.; Zhang, R.; Muldoon, M.; Watson, S.; Fischer, W.; Theiler, J.; Szinger, J.; Balachandran, H.; Buzby, A.; *et al.* Mosaic vaccines elicit CD8⁺ T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys. *Nat. Med.* **2010**, *16*, 324–328.
 139. Fischer, W.; Perkins, S.; Theiler, J.; Bhattacharya, T.; Yusim, K.; Funkhouser, R.; Kuiken, C.; Haynes, B.; Letvin, N.L.; Walker, B.D.; *et al.* Polyvalent vaccines for optimal coverage of potential T-cell epitopes in global HIV-1 variants. *Nat. Med.* **2007**, *13*, 100–106.
 140. Fischer, W.; Liao, H.X.; Haynes, B.F.; Letvin, N.L.; Korber, B. Coping with viral diversity in HIV vaccine design: A response to Nickle *et al.* *PLoS Comput. Biol.* **2008**, *4*, e15.
 141. Doria-Rose, N.A.; Learn, G.H.; Rodrigo, A.G.; Nickle, D.C.; Li, F.; Mahalanabis, M.; Hensel, M.T.; McLaughlin, S.; Edmonson, P.F.; Montefiori, D.; *et al.* Human immunodeficiency virus type 1 subtype B ancestral envelope protein is functional and elicits neutralizing antibodies in rabbits similar to those elicited by a circulating subtype B envelope. *J. Virol.* **2005**, *79*, 11214–11224.
 142. Mullins, J.I.; Nickle, D.C.; Heath, L.; Rodrigo, A.G.; Learn, G.H. Immunogen sequence: The fourth tier of AIDS vaccine design. *Expert Rev. Vaccines* **2004**, *3*, S151–S159.
 143. Nickle, D.C.; Jensen, M.A.; Gottlieb, G.S.; Shriner, D.; Learn, G.H.; Rodrigo, A.G.; Mullins, J.I. Consensus and ancestral state HIV vaccines. *Science* **2003**, *299*, 1515–1518.
 144. Dahirel, V.; Shekhar, K.; Pereyra, F.; Miura, T.; Artyomov, M.; Talsania, S.; Allen, T.M.; Altfeld, M.; Carrington, M.; Irvine, D.J.; *et al.* Coordinate linkage of HIV evolution reveals regions of immunological vulnerability. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11530–11535.
 145. Letourneau, S.; Im, E.J.; Mashishi, T.; Brereton, C.; Bridgeman, A.; Yang, H.; Dorrell, L.; Dong, T.; Korber, B.; McMichael, A.J.; *et al.* Design and pre-clinical evaluation of a universal HIV-1 vaccine. *PLoS One* **2007**, *2*, e984.
 146. Rosario, M.; Bridgeman, A.; Quakkelaar, E.D.; Quigley, M.F.; Hill, B.J.; Knudsen, M.L.; Ammendola, V.; Ljungberg, K.; Borthwick, N.; Im, E.J.; *et al.* Long peptides induce polyfunctional T cells against conserved regions of HIV-1 with superior breadth to single-gene vaccines in macaques. *Eur. J. Immunol.* **2010**, *40*, 1973–1984.
 147. De Groot, A.S.; Rivera, D.S.; McMurry, J.A.; Buus, S.; Martin, W. Identification of immunogenic HLA-B7 “Achilles’ heel” epitopes within highly conserved regions of HIV. *Vaccine* **2008**, *26*, 3059–3071.
 148. Wilson, C.C.; McKinney, D.; Anders, M.; MaWhinney, S.; Forster, J.; Crimi, C.; Southwood, S.; Sette, A.; Chesnut, R.; Newman, M.J.; *et al.* Development of a DNA vaccine designed to induce

- cytotoxic T lymphocyte responses to multiple conserved epitopes in HIV-1. *J. Immunol.* **2003**, *171*, 5611–5623.
149. Kaufman, D.R.; Li, F.; Cruz, A.N.; Self, S.G.; Barouch, D.H. Focus and breadth of cellular immune responses elicited by a heterologous insert prime-boost vaccine regimen in rhesus monkeys. *Vaccine* **2012**, *30*, 506–509.
150. Rosa, D.S.; Ribeiro, S.P.; Cunha-Neto, E. CD4⁺ T cell epitope discovery and rational vaccine design. *Arch. Immunol. Ther. Exp.* **2010**, *58*, 121–130.
151. Ribeiro, S.P.; Rosa, D.S.; Fonseca, S.G.; Mairena, E.C.; Postol, E.; Oliveira, S.C.; Guilherme, L.; Kalil, J.; Cunha-Neto, E. A vaccine encoding conserved promiscuous HIV CD4 epitopes induces broad T cell responses in mice transgenic to multiple common HLA class II molecules. *PLoS One* **2010**, *5*, e11072.
152. Almeida, R.R.; Rosa, D.S.; Ribeiro, S.P.; Santana, V.C.; Kallas, E.G.; Sidney, J.; Sette, A.; Kalil, J.; Cunha-Neto, E. Broad and cross-clade CD4⁺ T-cell responses elicited by a DNA vaccine encoding highly conserved and promiscuous HIV-1 M-group consensus peptides. *PLoS One* **2012**, *7*, e45267.
153. Kulkarni, V.; Valentin, A.; Rosati, M.; Alicea, C.; Singh, A.K.; Jalah, R.; Broderick, K.E.; Sardesai, N.Y.; le Gall, S.; Mothe, B.; *et al.* Altered immunodominance hierarchy and increased T-cell breadth upon HIV-1 conserved element DNA vaccination in macaques. *PLoS One* **2014**, *9*, e86254.
154. Hanke, T. Conserved immunogens in prime-boost strategies for the next-generation HIV-1 vaccines. *Expert Opin. Biol. Ther.* **2014**, *14*, 601–616.
155. Mothe, B.; Llano, A.; Ibarondo, J.; Zamarreno, J.; Schiaulini, M.; Miranda, C.; Ruiz-Riol, M.; Berger, C.T.; Herrero, M.J.; Palou, E.; *et al.* CTL responses of high functional avidity and broad variant cross-reactivity are associated with HIV control. *PLoS One* **2012**, *7*, e29717.
156. Rolland, M.; Nickle, D.C.; Mullins, J.I. HIV-1 group M conserved elements vaccine. *PLoS Pathog.* **2007**, *3*, e157.
157. Kulkarni, V.; Valentin, A.; Rosati, M.; Rolland, M.; Mullins, J.I.; Pavlakis, G.N.; Felber, B.K. Vaccine Branch, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD, USA. Unpublished work, 2014.
158. Kopycinski, J.; Cheeseman, H.; Ashraf, A.; Gill, D.; Hayes, P.; Hannaman, D.; Gilmour, J.; Cox, J.H.; Vasan, S. A DNA-based candidate HIV vaccine delivered via *in vivo* electroporation induces CD4 responses toward the $\alpha 4\beta 7$ -binding V2 loop of HIV gp120 in healthy volunteers. *Clin. Vaccine Immunol.* **2012**, *19*, 1557–1559.
159. Kennedy, J.S.; Co, M.; Green, S.; Longtine, K.; Longtine, J.; O'Neill, M.A.; Adams, J.P.; Rothman, A.L.; Yu, Q.; Johnson-Leva, R.; *et al.* The safety and tolerability of an HIV-1 DNA prime-protein boost vaccine (DP6-001) in healthy adult volunteers. *Vaccine* **2008**, *26*, 4420–4424.
160. Goepfert, P.A.; Elizaga, M.L.; Sato, A.; Qin, L.; Cardinali, M.; Hay, C.M.; Hural, J.; DeRosa, S.C.; DeFawe, O.D.; Tomaras, G.D.; *et al.* Phase 1 safety and immunogenicity testing of DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. *J. Infect. Dis.* **2011**, *203*, 610–619.
161. Hayes, P.; Gilmour, J.; von Lieven, A.; Gill, D.; Clark, L.; Kopycinski, J.; Cheeseman, H.; Chung, A.; Alter, G.; Dally, L.; *et al.* Safety and immunogenicity of DNA prime and modified

- vaccinia ankara virus-HIV subtype C vaccine boost in healthy adults. *Clin. Vaccine Immunol.* **2013**, *20*, 397–408.
162. Gudmundsdotter, L.; Nilsson, C.; Brave, A.; Hejdeman, B.; Earl, P.; Moss, B.; Robb, M.; Cox, J.; Michael, N.; Marovich, M.; *et al.* Recombinant Modified Vaccinia Ankara (MVA) effectively boosts DNA-primed HIV-specific immune responses in humans despite pre-existing vaccinia immunity. *Vaccine* **2009**, *27*, 4468–4474.
163. Sandstrom, E.; Nilsson, C.; Hejdeman, B.; Brave, A.; Bratt, G.; Robb, M.; Cox, J.; Vancott, T.; Marovich, M.; Stout, R.; *et al.* Broad immunogenicity of a multigene, multiclade HIV-1 DNA vaccine boosted with heterologous HIV-1 recombinant modified vaccinia virus Ankara. *J. Infect. Dis.* **2008**, *198*, 1482–1490.
164. Bakari, M.; Aboud, S.; Nilsson, C.; Francis, J.; Buma, D.; Moshiri, C.; Aris, E.A.; Lyamuya, E.F.; Janabi, M.; Godoy-Ramirez, K.; *et al.* Broad and potent immune responses to a low dose intradermal HIV-1 DNA boosted with HIV-1 recombinant MVA among healthy adults in Tanzania. *Vaccine* **2011**, *29*, 8417–8428.
165. Borthwick, N.; Ahmed, T.; Ondondo, B.; Hayes, P.; Rose, A.; Ebrahimsa, U.; Hayton, E.J.; Black, A.; Bridgeman, A.; Rosario, M.; *et al.* Vaccine-elicited human T cells recognizing conserved protein regions Inhibit HIV-1. *Mol. Ther.* **2014**, *22*, 464–475.
166. Li, J.; Valentin, A.; Kulkarni, V.; Rosati, M.; Beach, R.K.; Alicea, C.; Bear, J.; Hannaman, D.; Reed, S.G.; Felber, B.K.; *et al.* HIV/SIV DNA vaccine combined with protein in a co-immunization protocol elicits highest humoral responses to envelope in mice and macaques. *Vaccine* **2013**, *31*, 3747–3755.
167. Jaworski, J.P.; Krebs, S.J.; Trovato, M.; Kovarik, D.N.; Brower, Z.; Sutton, W.F.; Waagmeester, G.; Sartorius, R.; D'Apice, L.; Caivano, A.; *et al.* Co-immunization with multimeric scaffolds and DNA rapidly induces potent autologous HIV-1 neutralizing antibodies and CD8⁺ T cells. *PLoS One* **2012**, *7*, e31464.
168. Vargas-Inchaustegui, D.A.; Tuero, I.; Mohanram, V.; Musich, T.; Pegu, P.; Valentin, A.; Sui, Y.; Rosati, M.; Bear, J.; Kulkarni, V.; *et al.* Humoral immunity induced by mucosal and/or systemic SIV-specific vaccine platforms suggest novel combinatorial approaches for enhancing responses. *Clin. Immunol.*, submitted for publication, 2014.
169. Boyer, J.D.; Robinson, T.M.; Kutzler, M.A.; Vansant, G.; Hokey, D.A.; Kumar, S.; Parkinson, R.; Wu, L.; Sidhu, M.K.; Pavlakis, G.N.; *et al.* Protection against simian/human immunodeficiency virus (SHIV) 89.6P in macaques after coimmunization with SHIV antigen and IL-15 plasmid. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 18648–18653.
170. Muthumani, K.; Bagarazzi, M.; Conway, D.; Hwang, D.S.; Manson, K.; Ciccarelli, R.; Israel, Z.; Montefiori, D.C.; Ugen, K.; Miller, N.; *et al.* A Gag-Pol/Env-Rev SIV239 DNA vaccine improves CD4 counts, and reduce viral loads after pathogenic intrarectal SIV(mac)251 challenge in rhesus macaques. *Vaccine* **2003**, *21*, 629–637.
171. Boyer, J.D.; Maciag, P.C.; Parkinson, R.; Wu, L.; Lewis, M.G.; Weiner, D.B.; Paterson, Y. Rhesus macaques with high levels of vaccine induced IFN-gamma producing cells better control viral set-point following challenge with SIV239. *Vaccine* **2006**, *24*, 4498–4502.

172. Haigwood, N.L.; Pierce, C.C.; Robertson, M.N.; Watson, A.J.; Montefiori, D.C.; Rabin, M.; Lynch, J.B.; Kuller, L.; Thompson, J.; Morton, W.R.; *et al.* Protection from pathogenic SIV challenge using multigenic DNA vaccines. *Immunol. Lett.* **1999**, *66*, 183–188.
173. Yin, J.; Dai, A.; Lecureux, J.; Arango, T.; Kutzler, M.A.; Yan, J.; Lewis, M.G.; Khan, A.; Sardesai, N.Y.; Montefiore, D.; *et al.* High antibody and cellular responses induced to HIV-1 clade C envelope following DNA vaccines delivered by electroporation. *Vaccine* **2011**, *29*, 6763–6770.
174. Von Gegerfelt, A.S.; Alicea, C.; Valentin, A.; Morrow, M.; van Rompay, K.K.; Ayash-Rashkovsky, M.; Markham, P.; Else, J.G.; Marthas, M.L.; Pavlakis, G.N.; *et al.* Long lasting control and lack of pathogenicity of the attenuated Rev-independent SIV in rhesus macaques. *AIDS Res. Hum. Retroviruses* **2006**, *22*, 516–528.
175. Pal, R.; Wang, S.; Kalyanaraman, V.S.; Nair, B.C.; Whitney, S.; Keen, T.; Hocker, L.; Hudacik, L.; Rose, N.; Cristillo, A.; *et al.* Polyvalent DNA prime and envelope protein boost HIV-1 vaccine elicits humoral and cellular responses and controls plasma viremia in rhesus macaques following rectal challenge with an R5 SHIV isolate. *J. Med. Primatol.* **2005**, *34*, 226–236.
176. Pal, R.; Kalyanaraman, V.S.; Nair, B.C.; Whitney, S.; Keen, T.; Hocker, L.; Hudacik, L.; Rose, N.; Mboudjeka, I.; Shen, S.; *et al.* Immunization of rhesus macaques with a polyvalent DNA prime/protein boost human immunodeficiency virus type 1 vaccine elicits protective antibody response against simian human immunodeficiency virus of R5 phenotype. *Virology* **2006**, *348*, 341–353.
177. Rasmussen, R.A.; Hofmann-Lehman, R.; Montefiori, D.C.; Li, P.L.; Liska, V.; Vlasak, J.; Baba, T.W.; Schmitz, J.E.; Kuroda, M.J.; Robinson, H.L.; *et al.* DNA prime/protein boost vaccine strategy in neonatal macaques against simian human immunodeficiency virus. *J. Med. Primatol.* **2002**, *31*, 40–60.
178. Koopman, G.; Mortier, D.; Hofman, S.; Mathy, N.; Koutsoukos, M.; Ertl, P.; Overend, P.; van Wely, C.; Thomsen, L.L.; Wahren, B.; *et al.* Immune-response profiles induced by human immunodeficiency virus type 1 vaccine DNA, protein or mixed-modality immunization: Increased protection from pathogenic simian-human immunodeficiency virus viraemia with protein/DNA combination. *J. Gen. Virol.* **2008**, *89*, 540–553.
179. Hel, Z.; Nacsa, J.; Trynieszewska, E.; Tsai, W.P.; Parks, R.W.; Montefiori, D.C.; Felber, B.K.; Tartaglia, J.; Pavlakis, G.N.; Franchini, G. Containment of simian immunodeficiency virus infection in vaccinated macaques: Correlation with the magnitude of virus-specific pre- and postchallenge CD4⁺ and CD8⁺ T cell responses. *J. Immunol.* **2002**, *169*, 4778–4787.
180. Hel, Z.; Tsai, W.P.; Thornton, A.; Nacsa, J.; Giuliani, L.; Trynieszewska, E.; Poudyal, M.; Venzon, D.; Wang, X.; Altman, J.; *et al.* Potentiation of simian immunodeficiency virus (SIV)-specific CD4⁺ and CD8⁺ T cell responses by a DNA-SIV and NYVAC-SIV prime/boost regimen. *J. Immunol.* **2001**, *167*, 7180–7191.
181. Hel, Z.; Tsai, W.P.; Trynieszewska, E.; Nacsa, J.; Markham, P.D.; Lewis, M.G.; Pavlakis, G.N.; Felber, B.K.; Tartaglia, J.; Franchini, G. Improved vaccine protection from simian AIDS by the addition of nonstructural simian immunodeficiency virus genes. *J. Immunol.* **2006**, *176*, 85–96.

182. Hutnick, N.A.; Myles, D.J.; Hirao, L.; Scott, V.L.; Ferraro, B.; Khan, A.S.; Lewis, M.G.; Miller, C.J.; Bett, A.J.; Casimiro, D.; *et al.* An optimized SIV DNA vaccine can serve as a boost for Ad5 and provide partial protection from a high-dose SIVmac251 challenge. *Vaccine* **2012**, *30*, 3202–3208.
183. Wilson, N.A.; Keele, B.F.; Reed, J.S.; Piaskowski, S.M.; MacNair, C.E.; Bett, A.J.; Liang, X.; Wang, F.; Thoryk, E.; Heidecker, G.J.; *et al.* Vaccine-induced cellular responses control simian immunodeficiency virus replication after heterologous challenge. *J. Virol.* **2009**, *83*, 6508–6521.
184. Casimiro, D.R.; Wang, F.; Schleif, W.A.; Liang, X.; Zhang, Z.Q.; Tobery, T.W.; Davies, M.E.; McDermott, A.B.; O'Connor, D.H.; Fridman, A.; *et al.* Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. *J. Virol.* **2005**, *79*, 15547–15555.
185. Wilson, N.A.; Reed, J.; Napoe, G.S.; Piaskowski, S.; Szymanski, A.; Furlott, J.; Gonzalez, E.J.; Yant, L.J.; Maness, N.J.; May, G.E.; *et al.* Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. *J. Virol.* **2006**, *80*, 5875–5885.
186. Suh, Y.S.; Park, K.S.; Sauermann, U.; Franz, M.; Norley, S.; Wilfingseder, D.; Stoiber, H.; Fagrouch, Z.; Heeney, J.; Hunsmann, G.; *et al.* Reduction of viral loads by multigenic DNA priming and adenovirus boosting in the SIVmac-macaque model. *Vaccine* **2006**, *24*, 1811–1820.
187. Liu, J.; Ewald, B.A.; Lynch, D.M.; Denholtz, M.; Abbink, P.; Lemckert, A.A.; Carville, A.; Mansfield, K.G.; Havenga, M.J.; Goudsmit, J.; *et al.* Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime-boost regimens in rhesus monkeys. *J. Virol.* **2008**, *82*, 4844–4852.
188. Mattapallil, J.J.; Douek, D.C.; Buckler-White, A.; Montefiori, D.; Letvin, N.L.; Nabel, G.J.; Roederer, M. Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. *J. Exp. Med.* **2006**, *203*, 1533–1541.
189. Mattapallil, J.J.; Hill, B.; Douek, D.C.; Roederer, M. Systemic vaccination prevents the total destruction of mucosal CD4 T cells during acute SIV challenge. *J. Med. Primatol.* **2006**, *35*, 217–224.
190. Letvin, N.L.; Huang, Y.; Chakrabarti, B.K.; Xu, L.; Seaman, M.S.; Beaudry, K.; Koriath-Schmitz, B.; Yu, F.; Rohne, D.; Martin, K.L.; *et al.* Heterologous envelope immunogens contribute to AIDS vaccine protection in rhesus monkeys. *J. Virol.* **2004**, *78*, 7490–7497.
191. Seaman, M.S.; Santra, S.; Newberg, M.H.; Philippon, V.; Manson, K.; Xu, L.; Gelman, R.S.; Panicali, D.; Mascola, J.R.; Nabel, G.J.; *et al.* Vaccine-elicited memory cytotoxic T lymphocytes contribute to Mamu-A*01-associated control of simian/human immunodeficiency virus 89.6P replication in rhesus monkeys. *J. Virol.* **2005**, *79*, 4580–4588.
192. Sun, Y.; Schmitz, J.E.; Buzby, A.P.; Barker, B.R.; Rao, S.S.; Xu, L.; Yang, Z.Y.; Mascola, J.R.; Nabel, G.J.; Letvin, N.L. Virus-specific cellular immune correlates of survival in vaccinated monkeys after simian immunodeficiency virus challenge. *J. Virol.* **2006**, *80*, 10950–10956.
193. Yamamoto, T.; Johnson, M.J.; Price, D.A.; Wolinsky, D.I.; Almeida, J.R.; Petrovas, C.; Nason, M.; Yeh, W.W.; Shen, L.; Roederer, M.; *et al.* Virus inhibition activity of effector memory CD8⁺ T cells determines simian immunodeficiency virus load in vaccinated monkeys after vaccine breakthrough infection. *J. Virol.* **2012**, *86*, 5877–5884.

194. Letvin, N.L.; Rao, S.S.; Montefiori, D.C.; Seaman, M.S.; Sun, Y.; Lim, S.Y.; Yeh, W.W.; Asmal, M.; Gelman, R.S.; Shen, L.; *et al.* Immune and genetic correlates of vaccine protection against mucosal infection by SIV in monkeys. *Sci. Transl. Med.* **2011**, *3*, doi:10.1126/scitranslmed.3002351.
195. Koup, R.A.; Roederer, M.; Lamoreaux, L.; Fischer, J.; Novik, L.; Nason, M.C.; Larkin, B.D.; Enama, M.E.; Ledgerwood, J.E.; Bailer, R.T.; *et al.* Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. *PLoS One* **2010**, *5*, e9015.
196. Kaur, A.; Sanford, H.B.; Garry, D.; Lang, S.; Klumpp, S.A.; Watanabe, D.; Bronson, R.T.; Lifson, J.D.; Rosati, M.; Pavlakis, G.N.; *et al.* Ability of herpes simplex virus vectors to boost immune responses to DNA vectors and to protect against challenge by simian immunodeficiency virus. *Virology* **2007**, *357*, 199–214.
197. Earl, P.L.; Wyatt, L.S.; Montefiori, D.C.; Bilska, M.; Woodward, R.; Markham, P.D.; Malley, J.D.; Vogel, T.U.; Allen, T.M.; Watkins, D.I.; *et al.* Comparison of vaccine strategies using recombinant env-gag-pol MVA with or without an oligomeric Env protein boost in the SHIV rhesus macaque model. *Virology* **2002**, *294*, 270–281.
198. Amara, R.R.; Patel, K.; Niedziela, G.; Nigam, P.; Sharma, S.; Staprans, S.I.; Montefiori, D.C.; Chenareddi, L.; Herndon, J.G.; Robinson, H.L.; *et al.* A combination DNA and attenuated simian immunodeficiency virus vaccine strategy provides enhanced protection from simian/human immunodeficiency virus-induced disease. *J. Virol.* **2005**, *79*, 15356–15367.
199. Robinson, H.L.; Montefiori, D.C.; Villinger, F.; Robinson, J.E.; Sharma, S.; Wyatt, L.S.; Earl, P.L.; McClure, H.M.; Moss, B.; Amara, R.R. Studies on GM-CSF DNA as an adjuvant for neutralizing Ab elicited by a DNA/MVA immunodeficiency virus vaccine. *Virology* **2006**, *352*, 285–294.
200. Zhao, J.; Lai, L.; Amara, R.R.; Montefiori, D.C.; Villinger, F.; Chennareddi, L.; Wyatt, L.S.; Moss, B.; Robinson, H.L. Preclinical studies of human immunodeficiency virus/AIDS vaccines: Inverse correlation between avidity of anti-Env antibodies and peak postchallenge viremia. *J. Virol.* **2009**, *83*, 4102–4111.
201. Barouch, D.H.; Liu, J.; Li, H.; Maxfield, L.F.; Abbink, P.; Lynch, D.M.; Iampietro, M.J.; SanMiguel, A.; Seaman, M.S.; Ferrari, G.; *et al.* Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* **2012**, *482*, 89–93.
202. Vaccari, M.; Boasso, A.; Ma, Z.M.; Cecchinato, V.; Venzon, D.; Doster, M.N.; Tsai, W.P.; Shearer, G.M.; Fuchs, D.; Felber, B.K.; *et al.* CD4⁺ T-cell loss and delayed expression of modulators of immune responses at mucosal sites of vaccinated macaques following SIV(mac251) infection. *Mucosal Immunol.* **2008**, *1*, 497–507.
203. Vaccari, M.; Mattapallil, J.; Song, K.; Tsai, W.P.; Hryniewicz, A.; Venzon, D.; Zanetti, M.; Reimann, K.A.; Roederer, M.; Franchini, G. Reduced protection from simian immunodeficiency virus SIVmac251 infection afforded by memory CD8⁺ T cells induced by vaccination during CD4⁺ T-cell deficiency. *J. Virol.* **2008**, *82*, 9629–9638.
204. Cox, J.H.; Ferrari, M.G.; Earl, P.; Lane, J.R.; Jagodzinski, L.L.; Polonis, V.R.; Kuta, E.G.; Boyer, J.D.; Ratto-Kim, S.; Eller, L.A.; *et al.* Inclusion of a CRF01_AE HIV envelope protein boost with a DNA/MVA prime-boost vaccine: Impact on humoral and cellular immunogenicity and viral load reduction after SHIV-E challenge. *Vaccine* **2012**, *30*, 1830–1840.

205. Virnik, K.; Hockenbury, M.; Ni, Y.; Beren, J.; Pavlakis, G.N.; Felber, B.K.; Berkower, I. Live attenuated rubella vectors expressing SIV and HIV vaccine antigens replicate and elicit durable immune responses in rhesus macaques. *Retrovirology*. **2013**, *10*, doi:10.1186/1742-4690-10-99.
206. Von Gegerfelt, A.S.; Rosati, M.; Alicea, C.; Valentin, A.; Roth, P.; Bear, J.; Franchini, G.; Albert, P.S.; Bischofberger, N.; Boyer, J.D.; *et al.* Long-lasting decrease in viremia in macaques chronically infected with simian immunodeficiency virus SIVmac251 after therapeutic DNA immunization. *J. Virol.* **2007**, *81*, 1972–1979.
207. Palma, P.; Romiti, M.L.; Montesano, C.; Santilli, V.; Mora, N.; Aquilani, A.; Dispinseri, S.; Tchidjou, H.K.; Montano, M.; Eriksson, L.E.; *et al.* Therapeutic DNA vaccination of vertically HIV-infected children: Report of the first pediatric randomised trial (PEDVAC). *PLoS One* **2013**, *8*, e79957.
208. Rodriguez, B.; Asmuth, D.M.; Matining, R.M.; Spritzler, J.; Jacobson, J.M.; Mailliard, R.B.; Li, X.D.; Martinez, A.I.; Tenorio, A.R.; Lori, F.; *et al.* Safety, tolerability, and immunogenicity of repeated doses of dermavir, a candidate therapeutic HIV vaccine, in HIV-infected patients receiving combination antiretroviral therapy: Results of the ACTG 5176 trial. *J. Acquir. Immune Defic. Syndr.* **2013**, *64*, 351–359.
209. Casazza, J.P.; Bowman, K.A.; Adzaku, S.; Smith, E.C.; Enama, M.E.; Bailer, R.T.; Price, D.A.; Gostick, E.; Gordon, I.J.; Ambrozak, D.R.; *et al.* Therapeutic vaccination expands and improves the function of the HIV-specific memory T-cell repertoire. *J. Infect. Dis.* **2013**, *207*, 1829–1840.
210. Lisziewicz, J.; Bakare, N.; Calarota, S.A.; Banhegyi, D.; Szlavik, J.; Ujhelyi, E.; Toke, E.R.; Molnar, L.; Lisziewicz, Z.; Autran, B.; *et al.* Single DermaVir immunization: Dose-dependent expansion of precursor/memory T cells against all HIV antigens in HIV-1 infected individuals. *PLoS One* **2012**, *7*, e35416.
211. Carcelain, G.; Autran, B. Immune interventions in HIV infection. *Immunol. Rev.* **2013**, *254*, 355–371.
212. Gudmundsdotter, L.; Sjodin, A.; Bostrom, A.C.; Hejdeman, B.; Theve-Palm, R.; Alaeus, A.; Lidman, K.; Wahren, B. Therapeutic immunization for HIV. *Springer Semin. Immunopathol.* **2006**, *28*, 221–230.