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Poxvirus vectors as HIV/AIDS vaccines in humans

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The RV144 phase III clinical trial with the combination of the poxvirus vector ALVAC and the HIV gp120 protein has taught us that a vaccine against HIV/AIDS is possible but further improvements are still needed. Although the HIV protective effect of RV144 was modest (31.2%), these encouraging results reinforce the use of poxvirus vectors as HIV/AIDS vaccine candidates. In this review we focus on the prophylactic clinical studies thus far performed with the more widely studied poxvirus vectors, ALVAC, MVA, NYVAC and fowlpox expressing HIV antigens. We describe the characteristics of each vector administered either alone or in combination with other vectors, with emphasis on the immune parameters evaluated in healthy volunteers, percentage of responders and triggering of humoral and T cell responses. Some of these immunogens induced broad, polyfunctional and long-lasting $CD4^+$ and $CD8^+$ T cell responses to HIV-1 antigens in most volunteers, with preference for effector memory T cells, and neutralizing antibodies, immune parameters that might be relevant in protection. Finally, we consider improvements in immunogenicity of the poxvirus vectors by the selective deletion of viral immunomodulatory genes and insertion of host range genes in the poxvirus genome. Overall, the poxvirus vectors have proven to be excellent HIV/AIDS vaccine candidates, with distinct behavior among them, and the future implementation will be dictated by their optimized immune profile in clinical trials.

Introduction

At the end of 2010, an estimated 34 million people were living with human immunodeficiency virus (HIV) worldwide. The number of people dying of acquired immune deficiency syndrome (AIDS)-related causes fell to 1.8 million, down from a peak of 2.2 million in the mid-2000s. There were 2.7 million new HIV infections and the HIV incidence has fallen in 33 countries, 22 of them in sub-Saharan Africa, the region most affected by the AIDS epidemic (www.unaids.org). Much of that success has come in the past two years after the rapid scale-up of access to antiretroviral treatment and the implementation of HIV prevention approaches. Male condoms are highly effective for HIV prevention, but consistent use is hindered by issues of consumer dissatisfaction, adherence, slippage/breakage, and lack of receptive-partner control. Other interventions including treatment of sexually transmitted infections (STIs),[1] male circumcision,[1] and use of a tenofovir (TFV) 1% microbicide vaginal gel[2] have shown clinical efficacy in reducing HIV acquisition. Despite the proven effectiveness of existing prevention approaches, less than one out of five people at high risk for HIV has access, and current prevention approaches are not practical for everyone, especially women.

For these reasons, the development of an effective HIV/AIDS vaccine represents the best long-term solution to eradicate the pandemic. We have known since the mid-1980s that the body's natural immune response to HIV infection is completely inadequate, but we still lack fundamental knowledge regarding the nature, quality, quantity and durability of immune responses that should be induced, the ideal antigens to include, how to overcome the sequence variability engendered by the error-prone HIV reverse transcriptase, or even whether preventive vaccine strategies should focus on protection from infection or from disease progression. In spite of all these gaps in understanding the correlates of protection in HIV infection, advances have been made on many fronts with the development of novel vectors, adjuvants and antigen design strategies as components of an HIV vaccine.

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HIV Efficacy Trials

During the 32 y since the discovery of HIV, only four efficacy trials have been performed. Two phase 3 gp120 vaccine trials were conducted, each with a bivalent combination of two strains of HIV gp120 protein formulated in Alum. The VAX004 and VAX003 studies were initiated in 1998 and 1999, respectively, and the results were reported in 2003. These gp120 subunit vaccines showed no significant impact on acquisition of HIV-1 infection and had no impact on plasma viremia or peripheral CD4 T cell count.[3,4] The Step trial was a phase IIb proof-of-concept study of MERCK´s Adenovirus 5 (Ad5)-vectored gag/pol/nef vaccine in a three-dose regimen in 3000 volunteers with varying levels of pre-existing immunity to Ad5. Whereas the vaccine was shown to stimulate strong T cell responses, it failed either to protect volunteers from acquisition of infection or to reduce viral load after infection. Post-hoc analyses of men enrolled in the study showed a larger number of HIV infections in the sub-group of vaccinated men who were Ad5 seropositive and uncircumcised compared with a comparable placebo group.[5-7] However, the lack of efficacy in the Step trial may be attributed to insufficient potency, antiviral activities and breadth of epitope recognition.

The last phase 3 trial conducted was the RV144. This study involved 16402 healthy volunteers at low risk of HIV infection in Thailand and evaluated the efficacy of a prime-boost regimen

using four doses of a recombinant canarypoxvirus (ALVAC) expressing the HIV gag, pro and env genes (vCP1521) along with recombinant gp120 protein with Alum adjuvant at the last two doses (the same gp120 used in the VAX003 study). The results of this study, released in 2009, showed that volunteers in the vaccine group acquired 31.2% fewer HIV-1 infections than those in the placebo group after three years of follow-up.[8] This modest efficacy, although not deemed adequate for licensing, was the first indication that a vaccine could protect against HIV-1 infection.

The prime-boost strategy used in the RV144 trial induced neither broadly neutralizing anti-serum nor broadly reactive cytotoxic T cell responses against HIV. Preliminary analyses of the immune responses demonstrated that binding antibodies to HIV-1 clade B and E gp120s were present in 99% of vaccinated subjects but titers waned approximately 90% over 20 weeks. Antibody-dependent cell-mediated cytotoxicity (ADCC) with HIV-1 clade B and E gp120-coated targets was detected in about 75% of vaccinees for clade B and 25% for clade E. As with binding antibodies, titers were not stable and waned over 20 weeks. Neutralizing antibodies (NAb) targeted a subset of HIV Tier 1 and 2 viruses but were less potent than the failed VAX003 and VAX004 trials.[9] Recently, it was reported that the lack of response to a vaccine designed to induce clade-specific NAb to HIV-1 was associated with the presence of certain HLA class II alleles and heterodimers in some Southeast Asians.[10] In addition, vaccine-induced $CD4^+$ lymphoproliferation was the most substantial detectable T cell response elicited by the vaccine regimen.[9]

At present, only two correlates of risk of infection have been derived from the RV144 study. The first statistically significant correlate was IgG antibodies that bind to HIV-1 V1/V2 region grafted on MuLv gp70 protein. This parameter correlated inversely with the rate of HIV-1 infection and may have contributed to protection. The second was plasma IgA antibodies that bind HIV-1 Env. These IgA antibody responses correlated directly with a 54% increased risk in HIV infection rate among vaccinated volunteers, suggesting that these antibody responses may mitigate the effects of potentially protective antibodies reducing the protective effect of the vaccine regimen.[11,12] Overall the results of the RV144 indicated that this vaccine combination was effective in preventing infection but it showed no effect on the levels of viremia and/or CD4 T cell count in vaccinated subjects in whom HIV-1 infection was subsequently diagnosed.

The results of three failed and one marginally successful trial could be interpreted to mean that antibodies alone or CD8 T cells alone are not effective, and that a combination of B cell, CD4 and CD8 T cell responses need to be elicited by the future vaccine candidates.

Poxviruses as HIV/AIDS Vaccines

The RV144 trial provided for the first time evidence that an HIV/AIDS vaccine can prevent HIV-1 infection and highlight that poxvirus vectors should be considered as one of the future HIV/AIDS vaccine candidate vectors.

Poxviruses, and in particular vaccinia virus (VACV), were among the first animal viruses to be investigated as gene transfer vectors. Recombinant gene expression by VACV was first demonstrated in 1982.[13,14] Since then, poxviruses have been successfully used for molecular biology studies of virus-host cell interactions, for in vitro production and functional characterization of proteins, as well as live vaccines and tools for vaccine research.[15,16] Several unique features make poxvirus recombinants excellent candidates as vaccine vectors: (1) The stability of freeze-dried vaccine,[17] its low cost, ease of manufacture and administration; (2) The cytoplasmic site of gene expression; (3) The packing flexibility of the genome, which allows large amounts of the genome to be lost or deleted and foreign DNA to be integrated (at least 25 Kb) without loss of infectivity[18]; (4) The ability to induce both antibody and cytotoxic T cell responses against foreign antigens with long-lasting immunity after a single inoculation; (5) The extensive preclinical and clinical experience reached and (6) the diminishing prevalence of vaccinia-experienced population due to the interruption of smallpox vaccination in the 1970s following its eradication. Despite these advantages, complications observed in young children and immunocompromised individuals during the Smallpox Eradication Program brought forth concerns regarding the safety of reintroduction of VACV as immunizing agent.[19,20] Therefore, one of the approaches undertaken to enhance the safety of VACV has been the development of highly attenuated strains, like modified vaccinia virus Ankara (MVA) or the Copenhagen-derived NYVAC, or the use of members of the genus avipoxvirus such as canarypox (CNPV) and fowlpox (FWPV) viruses. These live viral vector vaccines mimic viral infections hereby eliciting the appropriate innate danger signals to the adaptive immune system.[21] Additionally, these replication-defective viruses provide unique forms of viral vaccines that combine the safety of a killed virus vaccine with the immunogenicity of a live virus vaccine by expressing gene products within cells so the antigens can be presented efficiently by both MHC class I and class II pathways.[22] Hence, and because of their safety profile, the attenuated poxvirus strains are prime candidates for generation of recombinant virus-based vaccines against infectious diseases and cancer.[23-26] Next, we will describe the specific features of the most widely studied attenuated poxvirus vectors MVA, NYVAC, CNPV (ALVAC) and FWPV and we will summarize their use as HIV/AIDS vaccine candidates in different prophylactic clinical trials in humans.

MVA

MVA is a highly attenuated vaccinia virus derived from the chorioallantoid vaccinia virus Ankara (CVA), a Turkish smallpox vaccine, after more than 570 serial passages in primary chicken embryo fibroblasts (CEF).[27] The MVA genome has lost about 30 kb of DNA compared with the parental virus strain, with most of the deletions located at both ends of the viral genome, including deletions in genes nonessential for replication, such as genes encoding for immunomodulatory proteins that counteract host immune responses.[28] Therefore, MVA has a limited replication capacity in human and most mammalian cell types, where it can replicate, but infectious particles are not formed and therefore the infection does not spread to other cells.[21,29] Only in

some cell lines, as BHK-21 and CEF, MVA can produce viral progeny.[30] The main features of MVA are a highly attenuated phenotype, a safety profile, the capacity to express efficiently foreign genes and the ability to trigger specific strong immune responses to the heterologous antigens, as it was determined in different animal models and in humans.[21,23-25]

MVA vectors expressing different HIV-1 antigens have been tested in several clinical trials in humans to determine the safety, efficacy and immunogenicity profiles.[23-25] Nowadays, more than 30 prophylactic phase I/II clinical trials have been performed or are ongoing with MVA-based HIV vaccines administered alone or in a prime/boost combination with DNA vectors, as it is summarized in **Table 1** (see also: www.iavireport.org). In general, MVA-based HIV vaccines are safe and highly immunogenic. However, the immunogenicity results observed are quite heterogeneous and these differences depend on many parameters, such as the type and number of HIV-1 antigens expressed, the doses of vaccine used, the route of administration, the immunization protocol and the techniques used to analyze the vaccine-induced humoral and T cell responses.

The first phase 1 clinical trial with an MVA-based HIV vaccine candidate used MVA-HIVA (MVA expressing Gag from HIV-1 clade A and different immunodominant CD8 T cell epitopes), in a DNA prime/MVA boost immunization protocol, and the results obtained showed a modest immunogenicity against HIV-1 antigens, with low percentages of vaccinees with positive IFNγ ELISPOT responses,[31-35] although higher doses of the same vaccine elicited an increase in the immunogenicity.[35] However, new phase 1/2 clinical trials using other different MVA-based HIV vaccine candidates have demonstrated that MVA vaccines are highly immunogenic. For example, vaccination with three doses of MVA-B (MVA expressing Env and Gag-Pol-Nef as a fusion protein of clade B) in healthy volunteers induced broad, polyfunctional and long-lasting $CD4^+$ and $CD8^+$ T cell responses to HIV-1 antigens in most vaccinees, with preference for effector memory T cells and elicited Env-specific antibody responses in 95% of the volunteers.[36,37] Moreover, prime/boost immunization regimens using DNA (encoding Env of HIV-1 clades A, B and C; Gag of clades A and B and RT and Rev of clade B) and MVA-CMDR (expressing Env of HIV-1 clade E and Gag-Pol of clade A) induced strong HIV-specific T cell immune responses in 90–100% of the immunized volunteers,[38-40] in spite of pre-existing immunity to VACV.[41] The same prime/boost immunization regimen, but with two doses of MVA-CMDR, showed also high HIV-specific T cell immune responses in about 86–100% of the vaccinees.[39]

With the use of the most advanced methodologies in the characterization of the immune responses triggered by the vaccine candidates, as the polychromatic intracellular cytokine staining (ICS) assay, it has been shown that some MVA-based vaccines elicited mainly $CD4^+$ T cell responses,[38,40,42-45] while others induced preferentially $CD8^+$ T cell responses.[36,37,39] Furthermore, the specific responses detected against the HIV antigens included in the vaccines also differed, with some MVA-based vectors inducing preferentially Env responses, while others triggered Gag or GPN-specific responses.[36,37,42,45] Finally, most of the MVA-based HIV vaccines were able to induce antibodies against Env in a high number of vaccinees, some with neutralizing capacity.

NYVAC

The attenuated NYVAC strain was derived from a plaque-cloned isolate of the Copenhagen vaccine strain (VACV-COP) by the precise deletion of 18 Open Reading Frames (ORFs) implicated in the pathogenicity and virulence of Orthopoxviruses as well as in host-range regulatory functions governing the replication competency of this virus on cells derived from certain species. The resultant vector was proven to be highly attenuated since it failed to disseminate in immunodeficient mice, displayed a dramatically reduced ability to replicate on a variety of human tissue culture cells and was unable to produce infectious virus in humans.[49] Despite its limited replication in human and most mammalian cell types, NYVAC provides a high level of gene expression and triggers antigen-specific immune responses when delivered in animals and humans.[24]

The potential of recombinants based on NYVAC strain as vaccine carriers against HIV has been studied in some clinical trials using different administration approaches that are summarized in **Table 2**. Although various protocols have been applied to induce effective immune responses, the prime/boost strategy with heterologous vectors, but using NYVAC recombinants as the boosting immunogen, has proven to be the best choice to elicit high quality antigen-specific immune responses in healthy volunteers.[50-52] Combination of DNA and NYVAC recombinants, both expressing the HIV Env, Gag, Pol and Nef antigens from clade C, elicited antigen-specific T cell responses in 90% of vaccinees in contrast with the 33% of response obtained using NYVAC alone. The vaccine-induced T cell responses in volunteers that received the DNA prime/NYVAC boost regimen were broad, polyfunctional, durable and mostly directed against Env antigen. Moreover, this experimental protocol induced the homing of potentially protective HIV-specific $CD4^+$ and $CD8^+$ T cells in the gut, the port of entry of HIV and one of the major sites for HIV spreading and depletion of CD4 T cells.[53]

Avipoxviruses

Avipoxviruses (APVs) belong to the Chordopoxvirinae subfamily of the Poxviridae family. Although APV infections have been reported to affect over 232 species in 23 orders of birds, the knowledge of the molecular and biological properties of APV is largely restricted to canarypox virus (CNPV) and fowlpox virus (FWPV) for which full genome sequences are available.[55,56] Molecular comparisons indicate that CNPV and FWPV share 55–71% amino acid identity, with the shorter size of the FWPV genome due to the partial loss of genes, which may reflects its milder virulence. They both express homologous cellular genes with immunomodulatory functions, which might be responsible for their different virulence and host-range,[55] but CNPV shows a broader tissue tropism in the permissive avian hosts, generally associated with higher mortality rates[57] than FWPV. Both viruses

Table 1. Prophylactic clinical trials using MVA as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ICS: Intracellular cytokine staining; ND: Non-determined; NAb: Neutralizing antibodies; LPR: Lymphoprolipherative response

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	P: 2 x DNA-HIVA B: 2 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	Antibodies against p24 in 11% of vaccinees.	ELISPOT: Vaccine induced T cell responses in 89% of vaccinees.	31,34
I	2 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	Not detected	ELISPOT: Vaccine-induced T cell responses in 88% of vaccinees. Low magnitude.	31,34
I	P: 2 x DNA-HIVA B: 1 x MVA-HIVA 2 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	ND	ELISPOT: Vaccine-induced T cell responses in 50–100% of vaccinees. Short-lived. $CD4^+$ T cell responses > $CD8^+$ T cell responses (91% vs. 31%). LPR in 25–62% of vaccinees received prime/boost regimen.	46
I	2 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	ND	ELISPOT: Vaccine-induced T cell responses in 8% of vaccinees.	35
IIa	P: 2 x DNA-HIVA B: 2 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	ND	ELISPOT: Vaccine-induced T cell responses in 3–5% of vaccinees.	35
I	P: 1 x DNA-HIVA B: 1 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	Not detected	ELISPOT: Vaccine-induced T cell responses in 10–25% of vaccinees.	33
I/II	P: 1 x DNA-HIVA B: 1 x MVA-HIVA	Gag p24 and p17 fused to 25 overlapping $CD8^+$ T cell epitopes (clade A)	ND	ELISPOT: Vaccine-induced T cell responses in 10% of vaccinees.	32
I	P: 3 x DNA-HIVIS B: 1 x MVA-CMDR	P: Env (clades A, B and C); Gag (clades A and B); RT and Rev (clade B) B: Env (clade E); Gag-Pol (clade A)	Antibodies against Env and p24 in 2% and 56% of vaccinees, respectively.	ELISPOT: Vaccine-induced T cell responses in 92% of vaccinees (86% to Gag and 65% to Env). $CD4^+$ T cell responses > $CD8^+$ T cell responses. LPR in 92% of vaccinees.	40
I	P: 3 x DNA-HIVIS B: 1 x MVA-CMDR	P: Env (clades A, B and C); Gag (clades A and B); RT and Rev (clade B) B: Env (clade E); Gag-Pol (clade A)	Antibodies against Gag in 40% and 76.4% of vaccinees, with and without pre-existing immune responses to VACV, respectively. Antibodies against Env in 2.7% of vaccinees.	ELISPOT: Vaccine-induced T cell responses in 100% of vaccinees, with and without pre-existing immune responses to VACV. Moderately lower magnitude of vaccine-induced T cell responses in vaccinees with pre-existing immune responses to VACV. LPR in 95% and 100% of vaccinees, with and without pre-existing immune responses to VACV, respectively. Higher magnitude of LPR in vaccinees without pre-existing immune responses to VACV.	41
I	P: 3 x DNA-HIVIS B: 1 x MVA-CMDR	P: Env (clades A, B and C); Gag (clades A and B); RT and Rev (clade B) B: Env (clade E); Gag-Pol (clade A)	ND	LPR in 100% of vaccinees ($CD4^+$ > $CD8^+$; 86.8% vs. 21%).	38
I	3 x MVA-CMDR	Env (clade E); Gag-Pol (clade A)	Antibodies against Env and p24 in 90% and 100% of vaccinees, respectively.	ELISPOT: Vaccine-induced T cell responses in 90% of vaccinees (Env > Gag; 90% vs. 40%). Low magnitude. ICS: Polyfunctional $CD8^+$ and $CD4^+$ T cell responses in 30% and 90% of vaccinees, respectively. Mainly directed against Env. Memory T cell responses in 60% of vaccinees. LPR in 100% of vaccinees.	42
I/II	P: 3 x DNA-HIVIS B: 2 x MVA-CMDR	P: Env (clades A, B and C); Gag (clades A and B); RT and Rev (clade B) B: Env (clade E); Gag-Pol (clade A)	Antibodies against Env in 90% of vaccinees. HIV-1 NAb in 31% (against clade B) and 83% (against clade CRF01_AE) of vaccinees.	ELISPOT: Vaccine-induced T cell responses in 97% of vaccinees (Gag > Env). ICS: $CD8^+$ and $CD4^+$ T cell responses in 59% and 55% of vaccinees, respectively. LPR in 100% of vaccinees.	39

Table 1. Prophylactic clinical trials using MVA as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ICS: Intracellular cytokine staining; ND: Non-determined; NAb: Neutralizing antibodies; LPR: Lymphoprolipherative response (continued)

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	3 x TBC-M4	Env, Gag, Tat-Rev and nef-RT (clade C)	Antibodies against Env in 100% of vaccinees. No HIV-1 NAb.	ELISPOT: Vaccine-induced T cell responses in 87–91% of vaccinees (mainly against Env and Gag). Modest magnitude.	47
I	3 x ADMVA	Env, Gag-Pol, Nef-Tat (clade B/C)	Antibodies against Env in 77% of vaccinees. HIV-1 NAb in 83% of vaccinees.	ELISPOT: Vaccine-induced T cell responses in 62% of vaccinees (Env > Pol and Nef-Tat). Low magnitude. ICS: HIV-specific T cell responses undetectable.	45
I	P: 2 x DNA B: 2 x MVA/HIV62 (DDMM) P: 1 x DNA B: 2 x MVA/HIV62 (DMM) 3 x MVA/HIV62 (MMM)	P: Gag, PR, RT, Env, Tat, Rev and Vpu (clade B) B: Gag, PR, RT, Env (clade B)	Antibodies against Env in 73% (DDMM) and 96.6% (MMM) of vaccinees. HIV-1 NAb in 7% (DDMM) and 30% (MMM) of vaccinees.	DDMM: $CD4^+$ T cell responses in 77% of vaccinees (evenly distributed between Env and Gag). $CD8^+$ T cell responses in 42% of vaccinees. Long-lasting $CD4^+$ and $CD8^+$ T cell responses in 38% of vaccinees. MMM: $CD4^+$ T cell responses in 43% of vaccinees (directed against Gag). $CD8^+$ T cell responses in 17% of vaccinees. Long-lasting $CD4^+$ and $CD8^+$ T cell responses in 8% and 4% of vaccinees, respectively. Polyfunctional responses (DDMM similar to MMM).	43
I	3 x MVA-B	Env, Gag-Pol-Nef (clade B)	Antibodies against Env in 95% of vaccinees. HIV-1 NAb in 33% of vaccinees.	ELISPOT: Vaccine-induced T cell responses in 75% of vaccinees, (Env > GPN > Gag). ICS: Polyfunctional $CD8^+$ and $CD4^+$ T cell responses in 92.3% and 69.2% of vaccinees, respectively. Memory T cell responses in 84.6% of vaccinees, with effector memory phenotype.	36,37
I	P: 2 x DNA-EP-1233 B: 2 x MVA-mBN32	21 cytotoxic T lymphocyte (CTL) and 18 helper T lymphocyte (HTL) epitopes from Gag, Pol, Vpr, Nef, Rev and Env.	ND	ELISPOT: Vaccine-induced T cell responses in 4% of vaccinees. ICS: Vaccine-induced T cell responses in 12% of vaccinees.	48

Table 2. Prophylactic clinical trials using NYVAC as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; NAb: Neutralizing antibodies. ICS: Intracellular cytokine staining; -: No data available

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	2 x NYVAC-C	Env, Gag-Pol-Nef (clade C)	Low levels of anti-gp140 antibodies in 15% of vaccinees.	ELISPOT: Vaccine-induced T cell responses in 50% of vaccinees. Not durable and mainly against Env.	52
I/II	2 x NYVAC-C	Env, Gag-Pol-Nef (clade C)	Low levels of IgG anti-gp140 antibodies in 27% of volunteers. Short-lived. No NAb.	ELISPOT: Vaccine-induced T cell responses in 33% of vaccinees. Not durable. ICS: HIV-specific T cell responses mediated by $CD4^+$ T cells and mainly directed against Env.	50,51
	P: 2 x DNA-C B: 2 x NYVAC-C		High levels of IgG anti-gp140 antibodies in 75% of vaccinees. Short-lived. No NAb.	ELISPOT: Vaccine-induced T cell responses in 90% of vaccinees. ICS: HIV-specific T cell responses mainly mediated by $CD4^+$ T cells and mostly against Env. Vigorous, broad, polyfunctional and durable.	
I/II	P: 3 x DNA-C B: 1 x NYVAC-C	Env, Gag-Pol-Nef (clade C)	-	ELISPOT: Vaccine-induced T cell responses in 91% of vaccinees.	54
	P: 2 x DNA-C B: 2 x NYVAC-C		-	ELISPOT: Vaccine-induced T cell responses in 80% of vaccinees.	

have been described as unable to replicate and disseminate infection in non-human and human primates,[58] but some studies have shown replication of FWPV in non-permissive mammalian cell cultures by the presence of infectious viral particles[59] or the occasional presence of immature forms and mature intracellular virus in infected cells.[60] However, a recent work demonstrated that despite the complete replication and detection of poxvirus mature virions by electron microscopy in FWPV-infected Vero cells, the new progeny was not infectious.[61] Although they do not replicate in mammals, CNPV and FWPV correctly express heterologous genes in human cells when used as recombinant vaccine vectors. However, the more advanced replication cycle, the longer transgene expression in human cells and the more balanced Th1/Th2 cytokine induction might confer to the FWPV-based recombinant vaccines the ability to induce a more effective immune response.[61]

Several APV-vectored vaccines have been licensed for commercial veterinary use against some animal infections,[26] demonstrating their efficacy as vaccine vectors. The following sections will address the use of CNPV and FWPV-vectored vaccines in the HIV/AIDS field.

Canarypox Virus (ALVAC)

ALVAC, a plaque-purified clone derived from an attenuated canarypox virus obtained from the wild-type strain after 200 passages in chick embryo fibroblasts, has been extensively evaluated in preclinical studies with non-human and human primates[62-66] and widely used in human clinical trials as an HIV/AIDS vaccine candidate.[67,68] Canarypox (ALVAC) vectors, the most extensively studied viral-based HIV vaccine followed by MVA, NYVAC and fowlpox, have been reported to be well tolerated and safe for humans[69,70] and to effectively prime the immune system for induction of antibodies and CD8 cell-mediated cytotoxicity by protein antigens. Several clinical trials using ALVAC-based HIV-1 recombinants expressing different HIV-1 antigens and administered alone or in a prime/boost combination with subunit protein vaccines or lipopeptides have been performed or are ongoing (**Table 3**; see also: www.iavireport.org).

Initial efforts have focused on products based on the HIV-1 envelope protein, since several Env epitopes have been described to induce NAb and cell-mediated immune responses. However, phase I clinical trials of candidate HIV/AIDS vaccines have confirmed that envelope subunit vaccines, although capable of inducing high titers of antibodies, were extremely inefficient in eliciting $CD8^+$ cytotoxic T lymphocytes (CTLs).[71-78] Second generation vaccines using live, recombinant, poxvirus constructs have proven to be far more potent CTL immunogens,[79-81] emphasizing the essential role of priming with a live recombinant vector for the induction of a CTL response. A number of trials of various subtype B canarypox-HIV vector primes and boosters with subunits gp120 or gp160 established the prime-boost strategy as a candidate for advanced testing.[71,82-85] Canarypox-based prime-boost regimens induced both cellular and humoral responses but $CD8^+$ responses on ELISPOT assay were low[84] and the presence of primary isolate neutralizing antibodies was not consistently detected.[86-90] These preliminary studies led to efficacy testing of this prime-boost regimen in a large (> 16,000 persons) trial initiated in October 2003 in Thailand. The phase III RV144 trial demonstrated a modest efficacy (31.2%) for prevention of HIV acquisition compared with placebo in a modified intention-to-treat analysis.[8] The results of this RV144 clinical trial indicated that the vaccine combination was effective in preventing infection but there was no significant difference in the mean viral load and/or in post-infection CD4 T cell counts among subjects who were found to have HIV infection in the vaccine group, as compared with those in the placebo group. Great efforts are being made into the deep characterization of the immune response generated by this vaccine regimen and in the identification of correlates of protection that could be further incorporated in the optimization of new poxvirus-HIV-based vaccine candidates.

Fowlpox Virus

FWPV-based vaccine candidates expressing HIV or SIV antigens have been successfully tested in different animal models using diverse immunization approaches. However, the best immune responses have been elicited in prime-boost combinations.[103-109] At present, only limited data are available on the use of FWPV-based HIV prophylactic vaccines in humans (**Table 4**). The conducted clinical trials have shown a good safety profile of the vector, but contrasting data have been obtained in humans, where their safety has not always been paralleled by high immunogenicity, as in macaques. The use of DNA prime expressing 65% of the HIV-1 genome including *gag*, *pol*, *env*, *tat* and *rev*, and recombinant FWPV boost contained *gag* and *pol*, all from clade B, was no immunogenic when tested in healthy volunteers enrolled in a phase I/IIa clinical trial.[110] The same results were obtained using identical immunization regimen, but with higher doses of DNA and FWPV vectors containing homologous HIV-1 clade A/E sequences.[111] Interestingly, these vaccines elicited both $CD4^+$ and $CD8^+$ T cell responses in non-human primate models.[104-106] Better immunogenic profiles were obtained recently in a phase I trial using different combinations of recombinant MVA and FWPV vectors containing matching HIV-1 inserts.[44] Although FWPV-HIV was poorly immunogenic when given alone, it significantly induced HIV-specific $CD4^+$ and $CD8^+$ T cell responses when used as a booster after two MVA-HIV doses. These results better correlated with the preclinical data in monkeys[109] and highlight that future evaluation of FWPV-vectored vaccines should be addressed in prime-boost regimens.

Preexisting immunity to viral vectors is a major issue for the development of viral-vectored vaccines. In addition to the general features of poxviruses, CNPV and FWPV do not elicit high levels of NAb against themselves, which allows the use of multiple doses of the vectors without affecting their potency. Moreover, CNPV and FWPV do not immunologically cross-react with orthopoxviruses and can be used in previously vaccinia-experienced individuals or in combination with other poxvirus strains, circumventing the potential neutralization of the vector.[91] In the context of preexisting immunity to poxvirus vectors, long-term persistence of vaccinia-specific memory T cells in vaccinated

Table 3. Prophylactic clinical trials using ALVAC as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ADCC: Antibody-dependent cell-mediated cytotoxicity; LPR: Lymphoprolipherative response; CTL: Cytotoxic T lymphocyte; ICS: Intracellular cytokine staining; NAb: Neutralizing antibodies; TM: Transmembrane; MN, LAI, SF-2, GNE8 and IIIB strains all from clade B

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	P: 2 x ALVAC-HIV (vCP125) B: 2 x rgp160	P: gp160 (HIV-1_{MN}) B: rgp160 (HIV-$1_{MN/LAI}$)	vCP125 alone did not elicit antibodies. NAb against MN isolate in 65% and 90% of the subjects after 1st and 2nd rgp160 boosters, respectively. Six months after the last boost, only 55% were still positive.	LPR: gp160-specific LPR in 25% of subjects after vCP125 injections and in 100% of volunteers after 1st booster and 12 mo after 1st injection. CTL: Env-specific $CD8^+$ CTL activity in 39% of volunteers and still present 2 y after initial immunization in 2/3 of subjects tested.	79,81
I	P: (2, 4) x ALVAC-HIV (vCP125) (effect of dose) B: (0, 2) x SF-2 rgp120	P: gp160 (HIV-1_{MN}) B: rgp120 (HIV-1_{SF-2})	vCP125 + rgp120 regimen elicited more frequent ($>$ 85%) HIV-1_{MN} and HIV-1_{SF-2} V3-specific antibodies. Fusion-inhibition antibodies to both HIV-1_{MN} and HIV-1_{SF-2} only in vCP125 + rgp120 recipients ($>$ 30%). HIV-1_{MN} and HIV-1_{SF-2} NAb in 100% of vCP125 + rgp120 recipients, in $<$ 65% of vCP125 alone recipients and in $>$ 55% of rgp120 alone recipients.	ADCC: Responses to HIV-1_{MN} and HIV-1_{SF-2} rgp120 targets more often (70%) in vCP125 high dose + rgp120 recipients after 4th injection. LPR: HIV-1_{LAI} or HIV-1_{MN} rgp160-specific LPR more often in vCP125 + rgp120 recipients 2 weeks after 4th immunization. CTL: For low-dose regimen, Env-specific CTL responses in 25% of subjects immunized with 2 or 3 injections of vCP125. For high-dose regimen, vCP125 + rgp120 regimen elicited $CD8^+$ CTL activity more often (37%) than immunization with vCP125 (22%) or rgp120 (10%) alone. Memory $CD8^+$ T cell response against HIV-1_{MN} rgp160 in 22% of vCP125 + rgp120 recipients. Reported cross-clade CTL reactivities.	80,91,92
I	P: 4 x ALVAC-HIV (vCP205) B: 2 x SF-2 rgp120	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: rgp120 (HIV-1_{SF-2})	vCP205 injections did not result in detectable V3 peptide binding or NAb to HIV-1_{MN} or HIV-1_{SF-2}. rgp120 additional boosts resulted in 100% volunteers exhibiting binding or NAb to both V3 peptides MN and SF-2. 87.5% volunteers developed NAb to the primary isolate BZ167 but to none of 8 other primary isolates.	Env/Gag-specific $CD8^+$ CTLs induced at least once in 64% of volunteers.	83
I	P: (0, 3, 5) x ALVAC-HIV (vCP205) B: (0, 3) x CLTB-36	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: p24E-V3 MN synthetic peptide	vCP205 induced low levels of NAb against MN strain in 33% of volunteers after 4th injection. NAb against a non-syncytium-inducing clade B primary isolate (Bx08) not detected. CLTB-36 peptide induced no NAb.	CTL activity in 33% of volunteers immunized with vCP205, mainly after 4th injection and directed against Env, Gag and Pol. CLTB-36 peptide induced no CTLs.	93
I	P: (3–6) x ALVAC-HIV (vCP205) B: 2 x SF-2 rgp120	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: rgp120 (HIV-1_{SF-2})	vCP205 + rgp120 regimen resulted in NAb to MN strain in 91% of subjects (rgp120 boost enhanced titer and frequency of NAb to HIV-1_{MN} from 70% to 91%).	vCP205 + rgp120 regimen elicited durable Env/Gag-specific $CD8^+$ T cell responses in 62% of subjects (rgp120 boost did not increase Env-$CD8^+$ CTL response). Cross-clade CTL reactivities reported.	85,92
II	P: 4 x ALVAC-HIV (vCP205) B: (0, 4) x SF-2 rgp120	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: rgp120 (HIV-1_{SF-2})	Binding antibodies to SF-2 antigen 2 weeks after 2nd, 3rd and 4th vaccinations in $<$ 5% of vCP205 alone recipients at all times and in 53%, 92% and 93% of vCP205 + gp120 recipients, respectively. NAb to MN strain in 94% of vCP205 + gp120 recipients and in 56% of subjects given vCP205 alone.	LPR: Proliferative responses in 28% of volunteers. Significantly more frequent Env-specific LPR among recipients of the combination vCP205 + rgp120 than among recipients of vCP205 alone. CTL: Env/Gag-specific $CD8^+$ CTLs in 33% of vaccinees at some time point.	94
I	P: (3–6) x ALVAC-HIV (vCP205) (high dose) B: (0–6) x SF-2 rgp120	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: rgp120 (HIV-1_{SF-2})	NAb to MN strain in 87% of vaccinees. rgp120 boost as the strongest predictor of NAb response. Significant increased NAb response in volunteers who received vCP205 followed by rgp120 compared with volunteers who received both vaccines simultaneously.	Number of doses of vCP205 as a significant predictor of CTL response. Frequency of CTL responses after 2, 3, 4, 5 and 6 doses was 19%, 30%, 42%, 42% and 18%, respectively. rgp120 boost did not affect CTL response. Vaccine regimen induced a durable CTL response.	95

Table 3. Prophylactic clinical trials using ALVAC as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ADCC: Antibody-dependent cell-mediated cytotoxicity; LPR: Lymphoprolipherative response; CTL: Cytotoxic T lymphocyte; ICS: Intracellular cytokine staining; NAb: Neutralizing antibodies; TM: Transmembrane; MN, LAI, SF-2, GNE8 and IIIB strains all from clade B (continued)

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	4 x ALVAC-HIV (vCP205)	gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI})	NAb to primary and cell line-adapted clade B strains in 10% and 15% of vaccinees, respectively. NAb responses against clades A and D not detected.	CTL: Clade B Env/Gag-specific CTL responses in 20% of vaccinees. ELISPOT: Clade B Env/Gag-specific $CD8^+$ T cell responses in 45% of vaccinees. Cross-reactivity against clade A or D antigens reported.	96
I	4 x ALVAC-HIV (vCP205) subcutaneously via ex vivo transfected autologous DCs (DC arm) 4 x ALVAC-HIV (vCP205) intradermally (i.d. arm) 4 x ALVAC-HIV (vCP205) intramuscularly (i.m. arm)	gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI})	ALVAC vector elicited strong responses in all vaccination groups. This response decreased but remained positive 18 mo after vaccination. gp160 responses were highest in i.m. arm and comparable in i.d. and DC arms, decreased over time and not detected 18 mo after vaccination. gag p24 responses were lowest in magnitude and not detected in DC arm.	LPR: Mainly mediated by CD4 T cells and most frequent in DC arm (57% of subjects responded to at least one of the HIV-1 antigens tested with 43% responding to all 3 antigens, AT-2 HIV, gp160 and p24) with at least 18-mo durability after the final vaccination. CTL: Env-specific CD8 CTL responses in 25% of subjects in i.m. arm. ELISPOT: Modest cellular CD8 responses in 29% of subjects in DC arm (gag-specific) and in 12.5% of subjects in i.m. arm (env-specific).	97
I	4 x ALVAC-HIV (vCP1452) (high vs. regular dose)	gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}); synthetic polypeptide encompassing several human *nef* and *pol* epitopes; E3 and K3 VACV proteins	46% of the high-dose recipients had low titers of binding antibodies to Gag compared with 14% observed in the regular-dose recipients. Six months after last vaccination antibody responses had decreased significantly (7% and 4% for high- and regular-doses, respectively). In both high- and regular-dose groups, low titers of NAb against HIV-1_{MN} in 92% of subjects.	Env/Gag-specific CTL responses in 8% of high-dose recipients and in 16% of regular-dose recipients.	98
II	P: 4 x ALVAC-HIV (vCP1452) B: (0, 2) x MN rgp120	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}); synthetic polypeptide encompassing several human *nef* and *pol* epitopes; E3 and K3 VACV proteins B: rgp120 (HIV-1_{MN})	Peak of anti-gag p24 and anti-gp120 binding antibodies in both vaccine groups after 4th vaccination. Anti-gag p24 responses between 2 vaccine groups not significantly different (60% in vCP1452 recipients vs. 47% in vCP1452 + rgp120 recipients). Combination regimen statistically significant higher responses to anti-gp120 (95% vs. 70% in vCP1452 alone). vCP1452 + rgp120 group had higher titers of NAb against MN strain than vCP1452 alone. In this group, NAb titers higher after 1 protein boost than after 2 boosts. Very weak neutralization activity against heterologous reference strains.	Cellular immune responses did not differ between vaccinees and placebos. The only exception was LPR after 4th vaccination, for which the net response rate to rgp120 in vCP1452 + rgp120 was statistically significant (26%).	99
II	P: 4 x ALVAC-HIV (vCP1452) B: (0, 2,3) x AIDSVAX B/B	P: gp120 (HIV-1_{MN}) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}); synthetic polypeptide encompassing several human *nef* and *pol* epitopes; E3 and K3 VACV proteins B: rgp120 (HIV-1_{MN}) + rgp120 (HIV-1_{GNE8})	Anti-gag p24 binding antibodies detected 2 weeks after the final vaccination in all vaccine groups with response rates ranging from 23% to 36% (no significant difference between vaccine groups). NAb to MN strain detected in all vaccine groups, with net responses ranging from 57% to 94%. Magnitude and frequency of NAb titers to HIV-1_{MN} higher in groups receiving rgp120 compared with vCP1452 alone.	Net cumulative HIV-specific $CD8^+$ IFN-γ ELISPOT assay responses (against env, gag, pol or nef) in 13% of vCP1452 alone recipients and in 16% of vCP1452 + rgp120 recipients.	84

Table 3. Prophylactic clinical trials using ALVAC as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ADCC: Antibody-dependent cell-mediated cytotoxicity; LPR: Lymphoprolipherative response; CTL: Cytotoxic T lymphocyte; ICS: Intracellular cytokine staining; NAb: Neutralizing antibodies; TM: Transmembrane; MN, LAI, SF-2, GNE8 and IIIB strains all from clade B (continued)

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I	P: (2, 4) x ALVAC-HIV (vCP300) B: (0, 2, 4) x SF-2 rgp120	P: gp120 (HIV-1_{MN}); TM domain of gp41 (HIV-1_{IIIB}); Gag and protease (HIV-1_{IIIB}); 3 CTL-dense regions of pol (HIV-1_{LAI}); 2 CTL-dense regions of nef (HIV-1_{LAI}) B: rgp120 (HIV-1_{SF-2})	Binding (MN gp120, MN V3 loop, SF-2 gp120, SF-2 gp120 V3 loop) or NAb (MN and SF-2 strains) more frequent and of higher titer in vCP300 + rgp120 recipients compared with vCP300 alone recipients. Simultaneous vaccination with vCP300 + rgp120 led to earlier development of an antibody response than sequential vaccination.	$CD8^+$ CTL responses in 61% of volunteers at any time point during the trial. 3–6 mo after last immunization (12 mo), 39% of all vaccinees had $CD8^+$ CTLs against at least 1 antigen. Durable response rates at the 12-mo time point were: 32% to *gag*, 22% to *env*, 19% to *pol* and 16% to *nef*.	100
I/II	P: 4 x ALVAC-HIV (vCP1521) B: (0, 2) x AIDSVAX B/E (2 different doses)	P: CRF01_AE gp120 (92TH023) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: CRF01_AE rgp120 (HIV-1_{A244}) + rgp120 (HIV-1_{MN})	95% of group 1 vaccinees (200 μg AIDSVAX B/E) had anti-MN and 86% had anti-A244 binding antibodies 2 weeks after 4th vaccination. 100% of group 2 vaccinees (600 μg AIDSVAX B/E) had anti-MN and 96% had anti-A244 binding antibodies at the same time. 47% of vCP1521 recipients positive for anti-p24 binding antibodies. NAb to MN or E strains in 98% or 71%, respectively, of vCP1521 + AIDSVAX B/E (high dose) subjects and in 100% or 47% of vCP1521 + AIDSVAX B/E (low dose) recipients.	ADCC: Activity to subtype B and to CRF01_AE in 96% and 84% of vCP1521 + AIDSVAX B/E volunteers, respectively (11% and 7% in placebo group). LPR: Responses to gp120 clade E or gp120 MN in 63% or 61% of volunteers, respectively. CTL: HIV-specific $CD8^+$ CTL responses against both subtype B gag/pol antigens and subtype E gp120 in 24% of the subjects.	89,101
I/II	P: 4 x ALVAC-HIV (vCP1521) B: 2 x oligomeric gp160 (ogp160) or 2 x bivalent gp120	P: CRF01_AE gp120 (92TH023) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: oligomerc gp160 (CRF01_AE gp120 strain 92TH023 + subtype B gp41 strain LAI) or bivalent gp120 (subtype B gp120 strain SF2 + CRF01_AE gp120 strain CM235)	Binding antibodies to CRF01_AE gp120 (92TH023) in 100% prime-boost subjects. Binding antibodies to p24 in 38% and 33% of gp120- or gp160-boosted subjects, respectively. NAb to CRF01_AE and subtype B laboratory strains in 95% of ogp160-boosted recipients and in 100% of gp120-boosted vaccinees, respectively.	LPR: HIV-specific responses in 84% of subunit-boosted recipients (10% of placebo subjects). 93%, 84% and 87% of gp160-boosted recipients developed specific responses to CM235, SF-2 and 92TH023, respectively; 68%, 75% and 55% of gp120 recipients proliferated to CM235, SF-2 and 92TH023, respectively. CTL: Cumulative HIV-specific $CD8^+$ CTL responses not statistically significant compared with those in placebo recipients.	90
III	P: 4 x ALVAC-HIV (vCP1521) B: 2 x AIDSVAX B/E	P: CRF01_AE gp120 (92TH023) linked to TM domain of gp41 (HIV-1_{LAI}); Gag and protease (HIV-1_{LAI}) B: bivalent gp120 (subtype B gp120 strain MN + CRF01_AE gp120 strain A244)	Binding antibodies to gp120 MN or gp120 A244 in 98.6% of vaccinees 6 mo after last immunization. Anti-p24 binding antibodies in 52.1% of vaccinees at the same time.	LPR: Proliferative responses against gp120 MN, gp120 A244 or p24 in 87.3%, 90.1% and 49.3% of vaccinees, respectively, 6 mo after the final dose. ELISPOT: Env/Gag-specific T cell responses in 19.7% of vaccinees 6 mo after the last immunization. ICS: Env-specific $CD4^+$ T cells in 34% of vaccinees.	8,102

Table 4. Prophylactic clinical trials using FWPV as an HIV/AIDS vaccine candidate. P: Prime; B: Boost; ND: Non-determined

Phase	Vaccine	Antigen	Humoral Responses	Cellular Responses	Ref.
I/IIa	P: 2 x DNA-HIV-B B: 1 x FPV-HIV-B	P: Gag, Pol, Env, Vpu, Tat and Rev (clade B) B: Gag and Pol (clade B)	ND	ICS : No differences between vaccine and placebo recipients for Gag or Pol-specific T cell immune responses.	110
I/IIa	P: 3 x DNA-HIV-AE B: 1 x FPV-HIV-AE	P: Gag, Pol, Rev, Tat, Env and Nef (clade A/E) B: Gag/Pol, Env, Tat/Rev (clade A/E)	No detected	ICS : No vaccine-induced $CD4^+$ or $CD8^+$ T cell responses.	111
I	5 x FPV-HIV	Env/Gag, Tat/Rev/Nef-RT (clade B)	Binding anti-p24 antibodies in 14.3% of vaccinees only after 3 doses. No anti-gp120 response. Short-lived.	ICS: Poorly immunogenic. The highest HIV-specific T cell response rate detected after 3 doses (17% in $CD4^+$ T cells). Short-lived.	44
	P: 2 x MVA-HIV B: 3 x FPV-HIV		Binding anti-p24 and anti-gp120 antibodies in about 37% and 17% of vaccinees, respectively, after 3 and 4 doses. Rarely detected at the end of the study.	ELISPOT: Vaccine-induced T cell responses in 65% of vaccinees. ICS: Polyfunctional $CD4^+$ and $CD8^+$ T cell responses in about 50% of vaccinees after 4 doses. Long-lasting $CD4^+$ and $CD8^+$ T cell responses in 29% and 42% of vaccinees, respectively.	
	5 x MVA-HIV		Binding anti-p24 and anti-gp120 antibodies in about 20% and 67% of vaccinees, respectively, after 3 and 4 doses. Rarely detected at the end of the study.	ELISPOT: Vaccine-induced T cell responses in 46% of vaccinees. ICS: Polyfunctional $CD4^+$ and $CD8^+$ T cell responses in 40% and 21% of vaccinees, respectively, after 4 doses. Long-lasting $CD4^+$ and $CD8^+$ T cell responses in 14% of vaccinees.	

individuals has been reported.[112] This study showed that proliferative vaccinia-specific memory responses persisted in 72.5% of the vaccinees and are not influenced by the time since priming or vaccine recalls. IFN-γ vaccinia-specific effector memory responses were detected in only 20% of the subjects, declined 45 y after priming independently of recalls and are always associated with a proliferative memory response. It has also been demonstrated that re-vaccination boosted both IFN-γ and proliferative responses independently of the time since priming.[112] However, the key issue is to determine the effect of such preexisting immunity to the vaccinia vector on the immunogenicity and efficacy of a poxvirus-based HIV vaccine. In this regard, it has been recently reported that preexisting immunity to vaccinia vector decreased SIV-specific CD8 and CD4 T cell responses but preserved SIV-specific humoral immunity and efficacy of a DNA/MVA vaccine in the rhesus macaque model using a pathogenic intrarectal SIV251 challenge.[113] The impact of preexisting immunity to vaccinia virus on the immunogenicity against HIV-1 antigens has also been evaluated after immunization of healthy volunteers with an HIV-1 DNA prime/MVA boost vaccine.[41] The results of this study showed that preexisting immunity to vaccinia vector decreased the magnitude of the responses but not the proportion of HIV-1 responders.[41]

Optimization of Poxvirus Vector-Based HIV/AIDS Vaccine Candidates: Deletion of Immunomodulatory Genes and Attenuated Replication Competent Vectors

The phase III RV144 clinical trial in Thailand using poxvirus vector ALVAC plus AIDSVAX (two gp120 proteins) showed for the first time that an HIV vaccine can prevent HIV infection.[8] Although the protective effect was modest (31.2%), these encouraging results reinforce the use of poxvirus vectors as HIV/AIDS vaccine candidates. However, novel, more efficient and optimized poxvirus vector-based HIV vaccines with the ability to enhance the magnitude, breadth, polyfunctionality and durability of the immune responses to HIV-1 antigens, together with an induction of NAb, are desirable. Thus, in an effort to generate optimized poxvirus vector-based HIV vaccines, several approaches are followed. Among them, we will focus in the removal of selected immunomodulatory genes in the VACV genome of MVA and NYVAC and in the generation of attenuated replication competent viruses.[23-25]

Although attenuated poxvirus vectors, as MVA and NYVAC, have deletions in several genes compared with their parental strains,[28,49] they still retain several immunomodulatory genes encoding proteins that can interfere with host immune responses, whose deletion may result in an increased immunogenicity against the foreign antigens.[114,115] The deletion of these genes would improve vaccine safety and immunogenicity because the immune system will now be activated more readily and will be able to detect and eliminate virions and virus-infected cells more efficiently. Therefore, MVA and NYVAC poxvirus vector-based HIV vaccines with deletions in single and multiple immunomodulatory VACV genes which antagonize host specific immune responses have been generated and the overall results obtained in

preclinical studies showed an important immunological benefit with a significant enhancement in the immunogenicity against HIV antigens compared with their parental poxvirus-based HIV vaccines.[116-119] For example, new optimized MVA-B HIV/AIDS vaccine candidates with single or multiple deletions in certain VACV immunomodulatory genes were able to induce in mice after a DNA prime/poxvirus boost immunization protocol a significant increase in the magnitude, quality and durability of $CD4^+$ and $CD8^+$ HIV-specific T cell responses, and in the antibody responses against gp120, when compared with the parental MVA-B.[116,117] Interestingly, these MVA-B deletion mutants induced $CD8^+$ T cell responses mainly directed against GPN, compared with the parental MVA-B in which $CD8^+$ T cell responses were mainly directed against Env and Gag. A more evenly distributed immune response has been reported with an MVA-C recombinant expressing Env and GPN of HIV clade C with a deletion of the viral gene encoding IL-18 binding protein.[120]

Regarding NYVAC-based vectors, NYVAC-C HIV/AIDS vaccine candidates (expressing clade C HIV antigens) with single or multiple deletions in certain VACV immunomodulatory genes that antagonize the IFN system, also showed enhanced immunogenicity in mice and expression of IFN and IFN-induced genes in DCs.[118,119,121] In addition, re-insertion of host-range genes *K1L* and *C7L* into the NYVAC-C vector-based HIV vaccine (termed NYVAC-C-KC) restores replication competence in human cells, but still retains a highly attenuated phenotype.[118,119] Finally, the HIV/AIDS vaccine candidate NYVAC-C with a combination of replication capacity in human cells and deletion of immunomodulatory genes that antagonize the IFN system has also been generated, with improved immunological features.[118,119]

Deletion of other VACV genes present in the genomes of MVA and NYVAC are being analyzed for further improvements in immunogenicity of these poxvirus vectors. It will be important to establish in future studies highly optimized MVA and NYVAC-based vectors that could be used alone or in combination with other immunogens as more potent vaccines.

Which Poxvirus Vector?

Taking into consideration all of the background information and the detailed characterization described in this review for the different poxvirus vectors, the results thus far obtained from many clinical trials in humans showed that poxviruses are on top of the iceberg to be considered as candidate immunogens in a future HIV/AIDS vaccine. However, not all the different poxvirus vectors behaved in a similar way or induced the same immunogenicity profile. These important differences have to be considered, and a critical analysis is necessary when selecting the right vector based on different criteria, such as safety, clinical benefits, immunogenicity profiles, and vector production for mass coverage, among other features. Further results obtained from new clinical trials will help to define which vector has more advantages as a final HIV/AIDS vaccine. Regarding safety, it is well established that all the different poxvirus vectors currently tested in clinical trials are safe, with minimal side effects particularly after receiving the first immunizing dose. Regarding clinical benefits, ALVAC is the poxvirus vector which has been used more times in clinical trials in humans, and until now it is the only one that has been tested in a phase III clinical trial (RV144) showing some efficacy. The results obtained from the RV144 trial are highly valuable, and some important correlates of protection have now been established. MVA is the next poxvirus vector more times analyzed in clinical trials, using different MVA-based HIV/AIDS vaccines. On the other hand, only one NYVAC-based HIV/AIDS vaccine (NYVAC-C) has been tested in prophylactic clinical trials, and few fowlpox-based HIV/AIDS vaccines are also available.

The immunogenicity profile elicited by the different poxvirus-based HIV/AIDS vaccine is one of the most important parameters to be considered in the choice of a future vaccine. Thus, are all poxvirus vectors activating similar immune mechanisms? From cell signaling studies, host cell gene activation pathways and type of immune responses induced by the poxvirus vectors, it is clear that each poxvirus vector triggers somehow similar but also distinct host cell signals and immune responses compared with the other vectors. The in vitro and in vivo properties of each vector must be taken into consideration when the choice of a poxvirus vector for clinical studies is under evaluation. Undoubtedly, there is a benefit when considering the poxvirus activation of long-term T cell memory responses and neutralizing capacity against HIV isolates. From the RV144 trial these immune parameters wane more rapidly than what has been observed in other clinical studies with MVA and NYVAC vectors. Since these vectors when administered as booster in combination with other vaccine candidates elicit broad immune responses against the target HIV antigen, the question is if we can improve the quantity and quality of both humoral and cellular immune responses by the type of protocol employed. Indeed, the efficacy observed in the RV144 trial strongly suggests that activation of both arms of the immune system will be needed for protection and that improved poxvirus vectors should be developed.

A question pending is the use of the poxvirus vectors to enhance antibody responses to HIV, which should be further explored. In fact, most studies with poxvirus vectors have been planned for activation of T cell responses to different HIV antigens, including Env. New forms of Env, with various degrees of glycosylation, containing all possible human B and T cell epitopes and/or directing them to activate B cells, can be engineered in the poxvirus genome and tested in animal models. Finding a form of Env capable of triggering broadly and sustainable neutralizing antibody responses against HIV should be the goal.

Overall, in view of the results obtained thus far with the different clinical trials involving poxvirus vectors, it is clear that these vectors fulfill many of the requirements we can consider important for an HIV/AIDS vaccine, like activating potent immune responses (humoral and cellular) that are broad, polyfunctional and durable of an effector phenotype. Moreover, the combination of ALVAC/gp120 induced antibodies of high avidity directed to the V1/V2 loop of Env. Therefore, which poxvirus vector will be used as a future HIV/AIDS vaccine will

depend on different immune parameters. While at present we cannot recommend one over another poxvirus vector, the immunological differences and similarities between MVA, NYVAC, ALVAC and Folwpox should be taking into consideration. Studies should aim to find out the best poxvirus vector or its combination with other immunogens, resulting on immune characteristics relevant for the control of HIV infection.

Concluding Remarks

The RV144 phase III clinical trial and its follow-up study in vaccinees has been instrumental for expansion of the HIV vaccine field, as the pursuit of an effective vaccine against HIV/AIDS is now regarded as a reachable goal within the next 10 y. Although we are still limited in the number of correlates of protection that are found associated with reduced incidence of infection in RV144 participants, current research with monkey models and clinical trials indicate that certain immune parameters, as activation of cellular (polyfunctional and effector memory $CD4^+$ and $CD8^+$ T cells) and humoral responses (binding antibodies to V1V2-loops and NAb against Tier 1 and 2 viruses) are good indicators for potential protective efficacy of a vaccine against HIV infection.

What have we achieved thus far? As reviewed here, new information on activation of specific immune responses to HIV antigens in various phase I/II clinical trials has emerged with the use of poxvirus vectors, either alone or in combination with other vectors. The different poxvirus platforms (ALVAC, MVA, NYVAC and fowlpox) are being intensively studied and we will learn more from the ongoing and planned clinical trials. It should be pointed out that in this review we only cover prophylactic and not therapeutic clinical trials with the poxvirus vectors, as more emphasis has been played in the HIV field in prophylactic vaccines.

Considering the immune responses triggered by the poxvirus vectors MVA and NYVAC in clinical trials, it becomes evident that these vectors are excellent HIV/AIDS vaccine candidates but further exploration is needed. In fact, improvements on the immune characteristics of these vectors have been achieved by the selective deletion of viral immunomodulatory genes still present in the genome of these viruses and by the incorporation of host range viral genes, giving them replication capacity in human cultured cells while maintaining the highly attenuated phenotype. The role of these genetic improvements in MVA and NYVAC will be known in the planned clinical trials.

In summary, in prophylactic clinical trials, the attenuated poxvirus vectors ALVAC, MVA, NYVAC and fowlpox have proven to be good activators of specific immune responses, and while the immune efficiency of each vector shows similarities and differences between them, in general both MVA and NYVAC are given higher immune response parameters than the other vectors. In the years ahead, we will see how these vectors gained further recognition as candidate HIV/AIDS vaccines. Which of the poxvirus vectors will be selected for future use, either alone or in combination with other vectors, protein components and/or adjuvants, will be defined in the next few years from results of the clinical trials. It will not be surprising if depending on the protocol of immunization and benefits, the selection of one vs. another of the poxvirus vectors described here is preferentially used in future HIV/AIDS vaccination programs.

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