

Drug evaluation: DNA/MVA prime-boost HIV vaccine

Stephen Kent*, Robert De Rose & Erik Rollman

Address

University of Melbourne
Department of Microbiology and Immunology
Melbourne
Victoria 3010
Australia
Email: skent@unimelb.edu.au

*To whom correspondence should be addressed

Current Opinion in Investigational Drugs 2007 8(2):159-167
© The Thomson Corporation ISSN 1472-4472

Oxford University and Nairobi University are jointly developing a HIVA DNA/modified vaccinia Ankara (MVA) prime-boost vaccine for the potential prevention of infection with HIV subtype A. The vaccination strategy consists of priming with a DNA vaccine made from HIV-1 clade A gag p24/p17 consensus sequence (pIHR HIVA) then boosting with a MVA virus expressing HIVA (MVA.HIVA). Phase II clinical trials of the vaccine are underway in Kenya and the UK.

Introduction

A vaccine is widely viewed as essential for ultimately controlling the global HIV epidemic, which has resulted in the death of more than 25 million individuals and the infection of approximately a further 40 million individuals worldwide [747372]. Both humoral and cellular immunity are desirable for an effective HIV vaccine, but induction of a broad neutralizing antibody response, the cornerstone of vaccine-mediated protection for most diseases, has so far proved elusive [747374]. Considerable evidence from primate [619857], [745506], [745509], [750183] and human studies [231784] suggests that T-cell immunity against HIV can slow progression to AIDS. Thus, although T-cell-based HIV vaccine strategies are unlikely to completely prevent infection, if such vaccines can reduce viral levels, slow disease progression and limit further transmission of the virus, this would be a quantum advance in reducing the burden of HIV/AIDS [750170].

Several vaccine strategies have been devised in order to induce HIV-specific T-cell immunity, the most successful of which have used viral vectors, often in combination with other modalities. The concept 'heterologous prime-boost vaccination' describes the sequential use of different vaccine modalities. It was first used to describe recombinant vaccinia virus vaccines boosted with recombinant protein vaccines in the early 1990s [245552], and a related approach (using a canarypox vaccine boosted by recombinant protein; ALVAC HIV vaccine, sanofi-aventis/Institut Pasteur/Walter Reed Army Institute of Research) is now undergoing phase III clinical trials in Thailand [587512]. However, when poxvirus vectors are used alone or in combination with protein vaccines, only a modest proportion of individuals (usually < 30%) develop detectable T-cell immunity, according to standard IFN γ enzyme-linked immunospot (ELISpot) measures of T-cell immunity [728357].

Vaccination with plasmid DNA vectors followed by a recombinant poxvirus boost was first described in the mid

Originator Oxford University, Nairobi University

Status Phase II Clinical

Indication HIV-1 infection

Actions DNA vaccine, Prophylactic vaccine, Recombinant viral vector vaccine

Synonyms HIV-1 clade A gag p24/p17 vaccine, HIVA DNA/MVA vaccine, HIV subtype A vaccine (prophylactic, DNA/MVA prime-boost), MVA HIV vaccine (prophylactic, prime-boost)

1990s as a potentially successful HIV vaccine strategy in mice by research groups at the University of Oxford [746509] and at universities in Australia [750077]; this approach showed considerable promise in primate studies in the late 1990s [333340], [745506]. Several research groups have moved these prime-boost approaches into clinical trials. The Oxford University/Nairobi University collaborative consortium led by Hanke and McMichael [720659] and sponsored by the International AIDS Vaccine Initiative (IAVI) is the most clinically advanced of the prime-boost approaches. This research group has moved a set of candidate recombinant DNA and attenuated vaccinia virus (MVA) vectors through several phase I and II clinical trials to establish their safety profile and T-cell immunogenicity in humans [720659].

The candidate DNA and MVA vaccine was developed against a subtype A HIV-1 immunogen termed HIVA, intended for prevention of HIV-1 infection or disease in Kenya and neighboring countries where subtype A HIV-1 is predominant [720663]. HIV strains are diverse worldwide and are divided into a number of broad subtypes (formerly termed 'clades'). It is widely believed that HIV vaccine antigens will be more effective immunogens if they more closely match circulating strains. HIVA is based on a consensus HIV-1 clade A sequence of the Gag protein coupled to a string of conserved cytotoxic T-lymphocyte (CTL) epitopes. The Gag proteins were chosen as immunogens because they are relatively conserved between strains. Overall, the design of the vaccine immunogens closely matches more than 70% of the HIV strains responsible for HIV-1 infection in Kenya [720663].

Primarily in an attempt to increase the immune breadth of the candidate vaccine, a second HIVA antigen - RENTA - was designed, which showed promise in preclinical studies [720658]. RENTA was inserted into the same vectors as HIVA, and is intended to be used in combination with HIVA in a four-component vaccine strategy [720658]. No clinical findings with the RENTA immunogen have been published as yet.

Synthesis and SAR

HIVA insert

The HIVA cassette contains a 5' insert of consensus HIV-1 subtype A gag and a 3' insertion of 23 partially overlapping

human CD8⁺ T-cell epitopes, many of which are conserved across multiple HIV subtypes [720663], [720674]. p17 and p24 sequences comprising 73% of *gag* were cloned in reverse order to the viral genome in order to facilitate Gag epitope processing and loading into MHC I complexes by avoiding the p17 N-terminal myristylated targeting of Gag to the cell membrane [720674]. Single murine and rhesus macaque CTL epitopes were also introduced to allow testing of the immunogenicity of the vaccine in animal models. The 3' polyepitope contains 8- to 10-mer CTL epitopes from Gag p24 and p17, Pol, Env and Nef proteins, identified in individuals in Kenya infected with subtype A HIV-1. Many of these CTL epitopes are conserved across multiple subtypes. In theory, each individual could, on average, respond to two or three epitopes across the HIVA immunogen. The entire HIVA insert was generated synthetically, with HIV-1 codons substituted for frequently used human codons in order to optimize for maximal expression in human cells. A Kozak consensus sequence was also included to ensure efficient initiation of translation [720674].

DNA vector

The pTHr vector used in clinical trials is a modified version of that used in preclinical testing [720663]. pTHr contains a pRc/CMV-derived plasmid backbone and polylinker, the human CMV promoter/enhancer/intron A and bovine growth hormone polyadenylation signal [720674]. Safety features include propagation of the plasmid in bacteria via a repressor-titration system (developed by Cobra Therapeutics Ltd), which obviates the need for an antibiotic resistance gene, and the absence of a mammalian origin of replication [720674]. In early reports, the plasmid was produced by 50-l batch fermentation of *Escherichia coli* strain DH1. DNA was purified from lysed cells by filtration and sequential anion exchange, gel permeation and ion pair chromatography [720662]. pTHr-HIVA for clinical testing was manufactured according to GMP by Cobra Therapeutics [720659].

MVA vector

MVA is a highly attenuated strain of vaccinia virus that was developed to prevent adverse effects associated with smallpox vaccination. The prototype MVA was developed by serial passage in chick embryo fibroblasts, resulting in deletion of approximately 15% of the vaccinia genome and an inability to replicate in most mammalian cells. MVA-571 (571 passages) has been used to vaccinate over 120,000 individuals since it was licensed in 1976, including those susceptible to complications associated with vaccinia vaccination, and has an excellent safety record. In the 1990s, MVA was developed for use as a vaccine vector [751961]. The MVA vector can accommodate large inserts, express these at high levels entirely within the cytoplasm, and can generate both antibody- and cell-mediated immune responses [751958]. The HIVA and RENTA immunogens are expressed under the control of the P7.5 promoter, having been stably inserted into the thymidine kinase locus of the MVA genome by standard homologous recombination techniques [751958]. The MVA vaccines were manufactured according to GMP guidelines by Impfstoffwerk Dessau-Tornau GmbH. MVA HIVA was cultured in primary

chicken embryo fibroblast cells, harvested by freeze-thawing, sonicated and purified by two successive steps of zonal centrifugation [720662].

RENTA insert

The RENTA immunogen was designed to complement the HIVA immunogen, broadening immune responses to subtype A HIV-1 by including regions derived from the Tat, reverse transcriptase (RT), gp41, Env and Nef proteins. The RENTA insert was cloned as a fusion protein into the pTHr plasmid, the same as that used to express the HIVA insert. For safety, *tat*, *RT* and *nef* sequences were altered to ensure expression of biologically inactive proteins, as confirmed by biological assays. Codon usage in the RENTA insert was optimized for expression in human cells in the same way as the HIVA insert [745754].

Preclinical development

Early polyepitope DNA and MVA vaccine development

Two initial reports in 1998 describe the immunogenicity of the first generation of polyepitope HIV-1 vaccine constructs, namely an engineered string of CD8⁺ T-cell epitopes made by the Oxford groups [315825], [510323], [720664], [746509]. The prototype DNA construct studied was termed pTHrHM and the vector-based system was named MVA.HM because it included CD8⁺ T-cell epitopes from both HIV-1 and malaria. Vaccination of BALB/c mice with each entity separately induced an immunogen-specific CTL response able to kill target cells [746509]. The capacity of human leukocyte antigen-matched human T-cell clones to recognize and kill human B-cell lines infected with prototype recombinant MVA constructs was confirmed *in vitro* [746509]. Most importantly, enhanced immunogenicity was observed when these two modalities were combined as a DNA-prime and protein-boost protocol [315825], [510278], [746509]. These early polyepitope constructs showed promising protective effects in malaria parasite-challenge systems [333344].

In a small number of rhesus macaque monkeys ($n = 3$), clearly detectable CD8⁺ T-cell immunity (1 to 5% of specific CD8⁺ T-cells measured by the MHC-tetramer method) was measured, and indications of DNA-prime MVA-boost enhancement were also observed [510278]. As the immunogen only expressed one known simian immunodeficiency virus (SIV) CTL epitope, the observed absence of protective effect after a subsequent intrarectal challenge with SIV_{mac251} was not surprising [510278]. A follow-up study in six macaques confirmed the induction of SIV-specific CD8⁺ T-cells after repeated DNA priming (up to five inoculations) [397680]. Clear immune enhancement was observed in the same animals following MVA boosting, with high frequencies of SIV-specific T-cells observed after the first MVA inoculation (5 to 20% of CD8⁺ T-cells detectable by tetramer); there was a lack of further enhancement above the initial response after a second and third MVA immunization, however, possibly explained by anti-MVA immunity [397680]. In another study in which ten macaques received prime-boost immunizations into the skin or mucosa followed by rigorous intrarectal challenge with

SIV_{mac239}, no actual protective effect was observed [745500]. This result contrasts with the findings of other researchers using whole gene-based DNA/poxvirus prime-boost approaches, which have conferred protection of macaques from challenge with various simian-human immunodeficiency viruses (chimeric viruses based on SIV but expressing an HIV envelope) [745506], [745509].

HIVA and RENTA in preclinical development

In the second-generation construct pTHHIVA, the prototype polyepitope immunogen was linked to the C-terminus of human codon-optimized Gag antigens p17 and p24, as described [720674]. This vaccine was constructed containing whole Gag proteins, as the importance of CD4+ T-cell help and breadth of immunity became better understood [720674], [745506], [745509]. Data from early studies in BALB/c mice showed that immunization with pTHr HIVA DNA (100 µg intramuscularly or two doses of 2 µg intradermally) or MVA HIVA (10⁷ plaque-forming units [pfu] intramuscularly) individually produced potent CTL-mediated lytic activity against target peptides, as determined in an *ex vivo* peptide restimulation assay [720674]. Furthermore, an interesting report on the kinetics of T-cell induction *in vivo* compared various routes of immunization with the pTHr HIVA DNA (100 µg) and MVA HIVA (5 × 10⁵ pfu) vaccine modalities in a mouse model in which naive T-cell receptor transgenic lymphocytes were transferred into a syngeneic recipient prior to vaccination. Following intravenous administration of the MVA HIVA vaccine, antigen-specific cell division was rapid, occurring mainly in the spleen. The response after intradermal injection was mainly in the cervical lymph nodes near the immunization site; intramuscular injection was least effective in inducing antigen-specific cell division, consistent with previous observations. For all routes of administration the proliferation response was localized and lasted no more than 7 days. Comparing the DNA and MVA vaccines revealed immunological imprinting and rapid induction by MVA, and a slower more sustained effect by the DNA vaccine [720654]. The role of CD4+ T-cell help in the immunogenicity of the vaccines has been described [720654]. Data from three macaques administered two priming injections of pTHr HIVA DNA (500 µg intramuscularly or 2 to 8 µg intradermally) and two boost injections with MVA HIVA (5 × 10⁸ pfu) – both the GMP-grade constructs – showed distinct T-cellular immunity, as detected by a validated IFN γ ELISpot assay. However, no protection was conferred to subsequent viral challenge in at least two of three animals [720664].

A second study reporting the immunogenicity of HIVA in primates was conducted in five macaques immunized with the GMP clinical trial vaccine batch [745754]. HIVA and RENTA were co-administered twice: 1 mg of each DNA plasmid was administered intramuscularly on days 1 and 4, followed by two intradermal immunizations with 5 × 10⁷ pfu of MVA.HIVA/RENTA on either days 8 and 12, or 20 and 24. Immunological results show barely detectable early cellular immunity *ex vivo* by intracellular cytokine staining (0.05 to 0.20% of T-cells were IFN γ +CD69+CD8+) after vaccination, and detectable, but low specific cellular

immunity (50 to 150 IFN γ spot-forming units (sfu)/10⁶ PBMCs) up to one year after vaccination [745754]. Furthermore, the same report demonstrates specific reactivity after *in vitro* culture of PBMCs with peptides and addresses the breadth of immunity. In summary, the immunogenicity of HIVA in macaques was reduced in comparison with that observed in mice [745754].

The RENTA immunogen was evaluated alone and together with the HIVA immunogen in a DNA prime-MVA boost protocol in both mice and macaques [720658]. Vaccination with RENTA induced CTLs in BALB/c mice and, somewhat surprisingly, acted additively when co-delivered with the HIVA immunogen, an unexpected finding speculated to be caused by unknown interactions at the local site of immunization. This study also addressed the issue of vaccine-elicited breadth of immunity within the RENTA immunogen in mice, seemingly illustrating clear dominance for a single epitope present in the N-terminal region of the RI protein. The responses in studies described by Nkolola and colleagues, obtained from five macaques vaccinated intramuscularly with pTHr RENTA DNA (25 µg intramuscularly) and MVA RENTA (10⁶ pfu), were limited in terms of detectable direct *ex vivo* T-cellular immunity. Responses were observed in only one of the five animals, and were detectable in PBMCs only after multiple weeks of *in vitro* peptide restimulation [720658]. However, the intra-immunogen epitope breadth was somewhat more balanced in the macaques than was measured in mice. In a more recent study, another five macaques received prime-boost immunizations, with marginally detectable cellular immunity measured *ex vivo*, but with specific reactivity demonstrated after PBMC culturing. The same study reports detectable responses after one year and discusses the breadth of immunity [745754].

Toxicity

DNA and MVA vaccines already have a proven track record for safety [751958]. DNA and rMVA vaccines encoding the HIVA immunogen, prepared according to GMP for the phase I clinical trials, were assessed for safety in 6- to 7-week-old BALB/c mice to gain approval for clinical testing [720662]. Groups of 20 mice were administered DNA vaccine (2 × 50 µg of pTHr HIVA by intramuscular injection) and MVA vaccine (2 × 10⁶ pfu of MVA HIVA intradermally), alone or in the prime-boost combination, or received empty vectors (as controls) to assess toxicity. A further 20 mice/group (6 as controls) were examined for vaccine persistence. These doses of DNA and MVA vaccines represent 1750- and 70-fold increases per body mass, respectively, compared with the human doses. All toxicological parameters (including clinical observations and measurements during the study, and post mortem, blood chemistry, food consumption and weight gain) were within normal parameters. Vaccine persistence was observed only at the site of administration beyond 5 weeks after the final immunization, consistent with vaccines of this type. The test vaccines were biologically active [720662]. There are currently no reports examining the toxicity of DNA and MVA constructs encoding the RENTA immunogen.

In order to study the therapeutic potential of the MVA HIVA vaccine in HIV-1 infected individuals receiving HAART, the vaccine was tested for safety in immunocompromised mice and SIV-infected rhesus macaques [720656]. This GLP study examined the safety, biodistribution and persistence of the MVA HIVA vaccine in severe combined immunodeficiency disease (SCID) mice and rhesus macaques immunocompromised by infection with SIV. Mice received two intradermal injections of 5×10^6 pfu of MVA HIVA, 15 days apart. Standard mouse toxicology tests did not reveal any vaccine-related toxicity. The vaccine persisted only at the site of injection, being detected in four out of six mice after 49 days, and was undetectable at all sites by day 81. A single dose of 5×10^7 pfu was well tolerated in macaques, with no vaccine-related pathology observed. MVA HIVA could no longer be detected 42 days after dosing [720656].

Metabolism and pharmacokinetics

No data are available on the metabolism of either vaccine. A murine biodistribution study is discussed in the toxicity section.

Clinical development

Phase I

Since 2000, a series of phase I/II clinical trials have been conducted in HIV-negative patients with the pTHr HIVA DNA vaccine alone, the MVA HIVA vaccine alone or the combination in a prime-boost regimen.

Three phase I clinical trials were initiated in 2000/2001 and reported by Mwau and colleagues [720659]. These clinical trials administered 0.1 to 0.5 mg of DNA vaccine intramuscularly to 18 individuals. After 9 to 14 months, 5×10^7 pfu of MVA HIVA vaccine was administered intradermally to nine of the same individuals. Both vaccines stimulated T-cell immunity in a high proportion of individuals, determined by measurements of cumulative positivity rate (ie, a positive IFN γ ELISpot response at any time during the clinical trial). Immunological responses were observed in all 8 individuals receiving the DNA/MVA prime-boost vaccines, in 14 out of 18 individuals administered DNA only, and in 8 out of 9 individuals receiving the MVA vaccine. At any given timepoint, the proportion of responders was significantly lower than the cumulative response rate. No marked differences were observed between the DNA doses, and T-cell responses to this vaccine were generally weak and transient. As with primate preclinical studies, MVA-induced responses were stronger and more frequent. Encouragingly, HIV-specific T-cell responses were detected by ELISpot assay after one year. Gag-specific antibody responses were induced in one individual [720659].

Data have also been published from three phase I clinical trials conducted in Kenya that evaluated the two vaccine components individually, and the prime-boost schedule in healthy, predominantly male, HIV-negative individuals [753139]. In the first study, pTHr HIVA DNA (500 μ g) or placebo was administered as two intramuscular injections,

21 days apart, to 13 individuals. Two intradermal injections of MVA HIVA (5×10^7 pfu) alone or placebo were tested in individuals ($n = 12$ and 6 , respectively) in the second clinical trial. The third trial enrolled ten individuals that had previously been primed with the DNA in the first study (> 32 weeks previously) to receive 5×10^7 pfu of MVA HIVA intradermally. Vaccine responses were assessed using *ex vivo* IFN γ ELISpot assays, with positive responses classified as being > 50 sfu/ 10^6 PBMCs above and at least twice background. Positive ELISpot responses in at least one peptide pool were recorded in 16, 25 and 10% of individuals in the DNA, MVA and prime-boost clinical trials, respectively [753139].

Data have been reported from a further double-blind, randomized phase I clinical trial evaluating higher doses of the DNA and MVA vaccines compared with placebo [720648]. A total of 24 healthy, HIV-negative individuals received two doses of pTHr HIVA DNA alone (4 mg intramuscularly) 4 weeks apart or placebo, or pTHr HIVA DNA followed 4 weeks later by two doses of MVA HIVA (10^8 pfu intradermally) or placebo. Using standard *ex vivo* IFN γ ELISpot assays, none of the eight MVA-only vaccinated individuals and four of the eight DNA plus MVA-vaccinated individuals responded. Additionally, two of the four responders in the DNA/MVA group had weak responses (36 and 103 sfu/ 10^6 PBMCs, respectively) that would be classified as negative in some settings. However, using a novel 'cultured ELISpot' assay to measure responses, all eight DNA/MVA-vaccinated individuals were considered to have responded and all four MVA-vaccinated individuals responded. CD4 $^+$ T-cells accounted for the majority of responses [720648]. Although these data suggest that low-level T-cell responses may have been primed by the vaccines, how these vaccines compare with other modalities is unclear, as this assay has not been employed in other phase I/II HIV vaccine clinical trials.

An additional set of three phase I trials in Kenya and Uganda were initiated in 2001 to 2002 in 10 to 18 individuals each, to generate further safety and immunogenicity data to support future trials. A brief summary of these trials was published recently and showed HIV-specific T-cell responses in a small proportion of responders (10 to 25%) using standard ELISpot assays [754700].

Phase II

Four larger phase I/II trials, each involving 50 to 119 volunteers, were initiated in 2002 and 2003 as a result of initial findings from phase I trials [753120]. A summary of findings has been published from trials in Kenya, in Uganda, and in Europe and South Africa (in 114, 50 and 81 individuals, respectively). Similarly disappointing levels of standard *ex vivo* ELISpot T-cell immune responses were seen: 3, 15 and 6%, respectively, in the prime-boost groups. There were no significant differences in the low response rates when different regimens or doses were used. According to this summary of the results, the responses detected were transient and occurred mainly after the first MVA boost [754700].

Interim data were published from a placebo-controlled, UK-based, phase I/II study evaluating early and late MVA booster schedules [749956]. A total of 120 HIV-negative individuals were randomized ($n = 40/\text{group}$) to receive two doses of pTHr HIVA DNA (0.5 or 2 mg intramuscularly) or placebo at weeks 0 and 4, followed by either early (weeks 8 and 12) or late (weeks 20 and 24) booster injections with MVA.HIVA (two doses of 5×10^7 pfu intradermally) or placebo. Of 119 evaluable individuals, 93% completed the course of vaccinations and were monitored for 52 weeks. Analysis of 1009 standard *ex vivo* IFN γ ELISpot data sets from 115 individuals showed positive immune responses in only 12% of individuals in the prime-boost group and 6% in the MVA only group. Neither of the two DNA vaccinations had a significant priming effect; most responses occurred after the first MVA vaccination for both the early- and late-boost regimens [749956].

Side effects and contraindications

A formal safety analysis has been reported from the first three phase I clinical trials of the two HIVA vaccines conducted in the UK in 18 individuals [720650]. Safety was assessed for the individual vaccine components, the pTHr HIVA DNA vaccine (two doses of 100 or 500 μg), the MVA HIVA vaccine (two doses of 5×10^7 pfu) and the combination DNA and MVA vaccines were administered twice with a 3-week interval. A cohort of nine individuals subsequently received two additional MVA HIVA boosters, 9 to 14 months later. Individuals were monitored for up to two years after the final vaccination. Two individuals discontinued the study because of an abnormal laboratory finding and a serious adverse event; however, these abnormalities were judged to be unrelated to the study vaccines. No moderate or severe local symptoms were observed after administration of the DNA vaccine, although most participants experienced injection-site reactions after receiving the MVA vaccine. Overall, both vaccine components and the prime-boost regimen were considered to be safe, well tolerated and suitable for progression into phase II clinical trials [720650].

In the Kenyan phase I study, the majority of reported adverse events were mild or moderate in severity and not vaccine related. Local reactogenicity was observed following the MVA, but not the DNA vaccination. No antinuclear antibodies or intercurrent HIV infections occurred [753139].

Further safety data were reported on the MVA HIVA vaccine. Sixteen HIV-1 infected individuals receiving HAART and with undetectable viral loads were administered two MVA HIVA boosters (4-week interval, 5×10^7 pfu intradermally). Eight of these individuals had received the pTHr HIVA DNA vaccine two years earlier. The vaccine was well tolerated and no severe adverse events were recorded [720649].

In the UK phase I/II study, local reactions occurred in 63 and 96% of the 119 individuals after the DNA-prime (3% were moderate) and MVA-boost (30% were moderate and 12% were severe) injections, respectively. Serious adverse events were reported in five individuals, although none

related to the vaccine. No severe laboratory abnormalities or systemic symptoms resulted from vaccinations [749956].

Patent summary

The HIVA vaccine was first disclosed in WO-00147955, assigned to the Medical Research Council (MRC), IAVI and the University of Nairobi. Equivalent applications, EP-01240186 and US-2003108562, are pending. The application claims an immunogen comprising HIV-1 gag p17/p24 and CTL epitopes; further claimed is the DNA/MVA prime-boost vaccination strategy.

In January 2001, the MRC, Nairobi University and IAVI agreed that they were to jointly own all existing and future patents covering the vaccine candidate [397054]. WO-2005047483, the only other application assigned to IAVI, MRC and Nairobi University, discloses artificial fusion proteins, including RENTA, and prime-boost strategies where at least one immunogen is RENTA.

Earlier applications filed by the University of Oxford relate to the underlying technology. The prime-boost strategy is proprietary to Oxxon Therapeutics Inc, a spinout company from Oxford University. However, Oxxon is not a collaborator in the development of HIVA [450943] or involved in the clinical development of prophylactic HIV vaccines WO-09856919, assigned to Isis Innovation Ltd (the commercialization arm of Oxford University) discloses a kit for generating a protective CD8 $^+$ T-cell immune response, including prime-boost vaccines. WO-00121201, also assigned to Isis, discloses the use of replication-deficient adenoviral vector, or MVA.

Current opinion

Immune priming with DNA and boost with a recombinant viral vector has been shown to efficiently induce vaccine-specific T-cell immune responses in several preclinical studies and in some clinical trials. The Oxford University research group has not yet demonstrated clearly that their approach confers protection in nonhuman primates, which would likely require generating a multi-epitope SIV construct equivalent to HIVA or RENTA.

There does not appear to be any negative interference between the HIVA and RENTA immunogens when delivered together; rather, the opposite was observed in mice, with actual enhancement of immunogenicity when delivered as a combination [720658]. This result is surprising, and encouraging, as many other investigations with similar vaccines show clear suppression of immunity when multiple components are delivered to one site simultaneously [745800]. Further preclinical and clinical evaluation of the HIVA and RENTA constructs together would be interesting.

The DNA/MVA prime-boost approach pioneered by the Oxford University research group proved safe and showed promising T-cell immunogenicity in initial phase I clinical trials, but was disappointing in larger phase I/II clinical trials using standard immunological evaluations. A study of higher doses of the vaccines showed clear priming of T-cell

responses in most individuals using a novel *in vitro*-cultured T-cell assay. The relevance and reproducibility of the low-level primed T-cell responses remains to be determined. The Oxford University research group reported that the DNA vaccine was less efficient at priming HIV-specific CD8+ T-cell responses, although the MVA vector can efficiently boost responses, particularly when efficiently primed by natural infection [754700]

It is unclear why poorer results were obtained in the larger phase I/II clinical trials. Improving the dose or antigen coverage (by adding the RENTA DNA and MVA vaccines) might improve immunogenicity rates in future studies, although the HIVA constructs should, if sufficiently immunostimulatory, have induced responses in most individuals. Low expression levels, lack of access of the vaccine antigens to secondary lymph node organs in humans or other unknown factors presumably limit the immunogenicity of the DNA and MVA vectors studied by this research group. IAVI initially sponsored the DNA/MVA clinical trials of the Oxford University consortium [374069], but in 2004, when results of the expanded phase I/II clinical trials were reported, it decided not to fund further trials [754704]

An alternative approach by Merck & Co Inc using adenovirus vectors demonstrated potent T-cell immunogenicity without *in vitro* culture in phase II clinical trials; this vaccine is now in expanded phase IIb clinical trials [436675], [746295]. A similar approach using DNA priming and adenovirus vector boosting from the NIH is also now in phase II trials [745508], [746507]. These latter two adenovirus-based approaches are currently perceived as the most advanced vaccines to test the concept of whether T-cell immunity can facilitate partial protection from HIV infection or disease.

Multiple investigator consortia worldwide are approaching the development of a prophylactic HIV vaccine similar to

HIVA/RENTA. A US research group based at Emory University led by Dr Harriet Robinson has focused on immunogens with whole HIV genes (*gag*, *pol* and *nef*) rather than the chimeric whole gene/epitope approach by the Oxford University consortia [745506], [746420]. Dr David Ho from the Aaron Diamond AIDS Research Center in the US is also developing subtype C DNA/MVA strategies for the Chinese population [745669]. Similarly, a Swedish research group led by Dr Britta Wahren used whole genes (*env*, *gag* and *pol*) as immunogens delivered with recombinant GM-CSF, and preliminary data reported at a vaccine conference demonstrated high rates of immunogenicity in a phase I clinical trial [745800]. An Australian research group used a DNA/fowlpoxvirus prime-boost approach that was poorly immunogenic in a phase I clinical trial, potentially because of the small doses used [745760], [746745]. Although the DNA/poxvirus prime-boost strategies are similar, there are many parameters that differ, including dose, antigens, adjuvants and administration route. Evaluation of the HIVA/RENTA DNA/MVA approach head-to-head with other related approaches would be of great interest.

In summary, the Oxford research group has pioneered the phase I/II clinical evaluation of a DNA/MVA prime-boost HIV-1 vaccine designed to induce T-cell immunity. To date, this approach has been disappointing in larger clinical trials. Other T-cell-based HIV vaccine approaches with recombinant adenovirus constructs have generated high levels of T-cell immunity in phase II clinical trials and are poised to be evaluated in large-scale trials. These clinical trials will be the first to test the concept that T-cell-based approaches can provide at least some protection from progressive HIV disease in humans, even if they do not substantially impact on rates of new infection. No HIV-1 vaccine has yet succeeded in producing broad neutralizing antibodies in clinical trials and this remains the largest obstacle for the HIV vaccine field.

Licensing

Nairobi University

By July 2000, Oxford University and Nairobi University had formed an alliance for the development of an anti-HIV vaccine. The R&D program was a collaboration between the research groups of Professor Andrew McMichael of the MRC Human Immunology Unit at Oxford University, UK and Professor JJ Bwayo of the University of Nairobi in Kenya [374069].

Development history

Developer	Country	Status	Indication	Date	Reference
Nairobi University	Kenya	Phase II	HIV-1 infection	01-APR-02	450493
Nairobi University	UK	Phase II	HIV-1 infection	01-APR-02	450493
Oxford University	Kenya	Phase II	HIV-1 infection	01-APR-02	450493
Oxford University	UK	Phase II	HIV-1 infection	01-APR-02	450493

Literature classifications

Chemistry

Study type	Result	Reference
HIVA design	HIVA contains a consensus HIV-1 subtype A p17 and p24 sequence and 23 partially overlapping 10-mer human CD8+ T-cell epitopes. The insert was generated synthetically and codon optimized for maximal expression in human cells.	720674
RENTA design	RENTA contains numerous HIV-1 subtype A regions from the Tat, RT, gp41, Env and Nef proteins. Sequences were altered to ensure expression of biologically inactive proteins and codon optimized for human expression.	745754

Chemistry (continued)

Study type	Result	Reference
DNA vector design	The pTHr vector contains a human CMV promoter/enhancer/intron A, a pRc/CMV-derived backbone and polylinker and bovine growth hormone polyadenylation signal. It is propagated in bacteria via a repressor-titration system and lacks a mammalian origin of replication.	720674
MVA vector design	MVA is a highly attenuated vaccinia virus. Immunogen inserts are expressed under the P7.5 promoter. MVA.HIVA was cultured in primary chicken embryo fibroblast cells, harvested by freeze-thawing, sonicated and purified by two successive steps of zonal centrifugation.	720662

Biology

Study type	Effect studied	Model	Result	Reference
<i>In vivo</i>	Immunogenicity	BALB/c mice immunized with PTHR HIVA DNA (100 µg intramuscularly or two doses of 2 µg intradermally) or MVA HIVA (10 ⁷ pfu intramuscularly).	Both vaccines induced potent CTL-mediated lytic activity against target peptides in <i>ex vivo</i> ELISpot assays.	720674
<i>In vivo</i>	Immunogenicity	Rhesus macaques (n = 3) administered two doses of both the pTHr HIVA DNA-prime (500 µg intramuscularly or 2 to 8 µg intradermally) and MVA HIVA boost (5 × 10 ⁶ pfu intradermally), with subsequent SIV _{mac251} challenge.	ELISpot assays showed potent immune responses to the vaccines.	720664
<i>In vivo</i>	Immunogenicity	Macaques (n = 5) vaccinated intramuscularly with repeated doses of pTHr RENTA DNA (1 mg intramuscularly) and MVA.RENTA (10 ⁶ pfu).	Responses were described in only one of the five animals, and were detectable in PBMCs only after multiple weeks of <i>in vitro</i> peptide restimulation.	720658
<i>In vivo</i>	Immunogenicity	Five macaques administered DNA prime (1 mg intramuscularly) and MVA boost (10 ⁶ pfu) with both HIVA and RENTA immunogens.	Low <i>ex vivo</i> T-cell immunity was seen, but cellular responses were detectable after long-term culture, up to one year later. No clear prime-boost effect was seen.	745754

Clinical

Effect studied	Model	Result	Reference
Safety and immunogenicity	Phase I, open-label, dose-escalation studies administering pTHr HIVA DNA vaccine (0.1 to 0.5 mg intramuscularly) to 18 individuals. After 9 to 14 months, 5 × 10 ⁷ pfu of MVA HIVA vaccine was administered intradermally to nine of the same individuals.	The cumulative IFN _γ ELISpot positivity rate showed immunological responses in all 8 individuals receiving the DNA/MVA prime-boost schedule, in 14 out of 18 individuals administered DNA only and in 8 of the 9 individuals receiving the MVA vaccine. Both vaccines were well tolerated, although MVA.HIVA caused injection-site reactions.	720659
Safety and immunogenicity	Three phase I clinical trials evaluating pTHr HIVA DNA (500 µg intramuscularly) or MVA HIVA (5 × 10 ⁷ pfu intradermally) vaccines, or DNA-prime-MVA-boost schedule, in healthy, predominantly male, HIV-negative individuals (n = 13, 12 and 10, respectively).	In IFN _γ ELISpot assays, positive responses in at least one peptide pool were recorded in 16, 25 and 10% of individuals, respectively. Adverse events were generally mild or moderate and not vaccine related.	753139
Immunogenicity	Phase I, double-blind, randomized clinical trial of pTHr HIVA DNA (two doses of 4 mg) plus either MVA.HIVA (two doses of 10 ⁶ pfu) or placebo in 24 healthy HIV-negative individuals (eight received vaccine and four received placebo in each group).	<i>Ex vivo</i> ELISpot assays revealed that none of the individuals responded in the MVA only group and four of the eight individuals responded in the DNA/MVA group. By cultured ELISpot, four out of eight individuals responded in the MVA only group and all eight individuals responded in the DNA/MVA group.	720648
Safety and immunogenicity	Phase I/II, placebo-controlled study evaluating two doses of pTHr HIVA DNA (0.5 or 2 mg intramuscularly) followed by early (weeks 8 and 12) or late (weeks 20 and 24) MVA boosters (two doses of 5 × 10 ⁷ pfu intradermally) in 120 HIV-negative individuals.	At one year, ELISpot data showed immune responses in 6 to 12% of individuals. Neither DNA vaccination had a significant priming effect; most responses occurred after the first MVA vaccination for both boost regimens. No severe adverse effects or abnormalities resulted from vaccinations.	749956
Safety	Phase I, open-label, dose-escalation studies administering one or two doses of pTHr HIVA DNA vaccine (0.1 to 0.5 mg intramuscularly) to 18 volunteers. After 9 to 14 months, MVA HIVA vaccine (5 × 10 ⁷ pfu intradermally) was administered to nine of the same individuals.	Both vaccine components and the prime-boost regimen were safe and well tolerated in individuals monitored for up to two years. Two discontinuations were judged to be unrelated to the vaccines. Injection site reactions occurred with the MVA, but not the DNA, vaccine.	720650

Associated patent

Title Methods and reagents for vaccination which generate a CD8 T-cell immune response

Assignee Isis Innovation Ltd

Publication WO-09856919 17-DEC-98

Priority GB-19979711957 09-JUN-97

Inventors McMichael AJ, Hill AVS, Gilbert SC, Schneider J, Plebanski M, Hanke T, Smith GL, Blanchard T

References

231784 Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD *J VIROL* 1994 68 7 4650-4655

245552 Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. Hu SL, Abrams K, Barber GN, Moran P, Zarling JM, Langlois AJ, Kuller L, Morton WR, Benveniste RE *SCIENCE* 1992 255 5043 456-459

315825 DNA multi-CTL epitope vaccines for HIV and *Plasmodium falciparum*: Immunogenicity in mice. Hanke T, Schneider J, Gilbert SC, Hill AV, McMichael A *VACCINE* 1998 16 4 426-435

333340 Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of consecutive priming with DNA and boosting with recombinant fowlpox virus. Kent SJ, Zhao A, Best SJ, Chandler JD, Boyle DB, Ramshaw IA *J VIROL* 1998 72 12 10180-10188

333344 Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. Schneider J, Gilbert SC, Blanchard TJ, Hanke T, Robson KJ, Hannan CM, Becker M, Sinden R, Smith GL, Hill AV *NAT MED* 1998 4 4 397-402

374069 First AIDS vaccine designed for Africa is cleared for testing in humans. IAVI's Scientific Blueprint 2000 seeks head-to-head trials and compressed timelines; with 'goal in sight,' IAVI urges US \$1.1 billion in new funding for focused R&D effort. The International AIDS Vaccine Initiative *PRESS RELEASE* 2000 July 11

397054 Trials of first AIDS vaccine candidate designed for Africa to begin. International AIDS Vaccine Initiative *PRESS RELEASE* 2001 January 27

397680 Induction of AIDS virus-specific CTL activity in fresh, unstimulated peripheral blood lymphocytes from rhesus macaques vaccinated with a DNA prime/modified vaccinia virus Ankara boost regimen. Allen TM, Vogel TU, Fuller DH, Mothe BR, Steffen S, Boyson JE, Shipley T, Fuller J, Hanke T, Sette A, Altman JD *et al J IMMUNOL* 2000 164 9 4968-4978

436675 Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Shiver JW, Fu TM, Chen L, Casimiro DR, Davies ME, Evans RK, Zhang ZQ, Simon AJ, Trigona WL, Dubey SA, Huang L *et al NATURE* 2002 415 6869 331-335

450943 Oxon Pharmaccines; products in development; key collaborations. Oxon Pharmaccines Ltd *COMPANY WORLD WIDE WEB SITE* 2002 May 14

510278 Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. Hanke T, Samuel RV, Blanchard TJ, Neumann VC, Allen TM, Boyson JE, Sharpe SA, Cook N, Smith GL, Watkins DI, Cranage MP, McMichael AJ *J VIROL* 1999 73 9 7524-7532

510323 Immunogenicities of intravenous and intramuscular administrations of modified vaccinia virus Ankara-based multi-CTL epitope vaccine for human immunodeficiency virus type 1 in mice. Hanke T, Blanchard TJ, Schneider J, Ogg GS, Tan R, Becker M, Gilbert SC, Hill AV, Smith GL, McMichael A *J GEN VIROL* 1998 79 1 83-90

587512 2004 sanofi-aventis results: Research and development. sanofi-aventis *COMPANY PRESENTATION* 2005 March 01

619857 Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Barouch DH, Santra S, Schmitz JE, Kuroda MJ, Fu TM, Wagner W, Bilska M, Craiu A, Zheng XX, Krivulka GR, Beaudry K *et al SCIENCE* 2006 290 486-492

720648 Induction of multifunctional human immunodeficiency virus type 1 (HIV-1)-specific T cells capable of proliferation in healthy subjects by using a prime-boost regimen of DNA- and modified vaccinia virus Ankara-vectored vaccines expressing HIV-1 Gag coupled to CD8+ T-cell epitopes. Goonetilleke N, Moore S, Dally L, Winstone N, Cebere I, Mahmoud A, Pinheiro S, Gillespie G, Brown D, Loach VR, J. Guimaraes-Walker A *et al J VIROL* 2006 80 10 4717-4728

720649 Expansion and diversification of virus-specific T cells following immunization of human immunodeficiency virus type 1 (HIV-1)-infected individuals with a recombinant modified vaccinia virus Ankara/HIV-1 Gag vaccine. Dorrell L, Yang H, Ondondo B, Dong T, di Gleria K, Suttill A, Conlon C, Brown D, Williams P, Bowness P, Goonetilleke N *et al J VIROL* 2006 80 10 4705-4716

720650 Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers. Cebere I, Dorrell L, McShane H, Simmons A, McCormack S, Schmidt C, Smith C, Brooks M, Roberts JE, Darwin SC, Fast PE *et al VACCINE* 2006 24 4 417-425

720654 Vaccine route, dose and type of delivery vector determine patterns of primary CD8+ T cell responses. Escourt MJ, Letourneau S, McMichael AJ, Hanke T *EUR J IMMUNOL* 2005 35 9 2532-2540

720656 Biodistribution and persistence of an MVA-vectored candidate HIV vaccine in SIV-infected rhesus macaques and SCID mice. Hanke T, McMichael AJ, Dennis MJ, Sharpe SA, Powell LAJ, McLoughlin L, Crome SJ *VACCINE* 2005 23 12 1507-1514

720658 Engineering RENTA, a DNA prime-MVA boost HIV vaccine tailored for Eastern and Central Africa. Nkolola JP, Wee EGT, Im E-J, Jewell CP, Chen N, Xu X-N, McMichael AJ, Hanke T *GENE THER* 2004 11 13 1068-1080

720659 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. Mwaou M, Cebere I, Sutton J, Chikoti P, Winstone N, Wee EGT, Beattie T, Chen YH, Dorrell L, McShane H, Schmidt C *et al J GEN VIROL* 2004 85 4 911-919

720662 Lack of toxicity and persistence in the mouse associated with administration of candidate DNA- and modified vaccinia virus Ankara (MVA)-based HIV vaccines for Kenya. Hanke T, McMichael AJ, Samuel RV, Powell LAJ, McLoughlin L, Crome SJ, Edlin A *VACCINE* 2002 21 1-2 108-114

720663 Development of a DNA-MVA/HIVA vaccine for Kenya. Hanke T, McMichael AJ, Mwaou M, Wee EGT, Cebere I, Patel S, Sutton J, Tomlinson M, Samuel RV *VACCINE* 2002 20 15 1995-1998

720664 A DNA/MVA-based candidate human immunodeficiency virus vaccine for Kenya induces multi-specific T cell responses in rhesus macaques. Wee EGT, Patel S, McMichael AJ, Hanke T *J GEN VIROL* 2002 83 1 75-80

720674 Design and construction of an experimental HIV-1 vaccine for a year-2000 clinical trial in Kenya. Hanke T, McMichael AJ *NAT MED* 2000 6 9 951-955

728357 High-dose recombinant canarypox vaccine expressing HIV-1 protein, in seronegative human subjects. Goepfert PA, Horton H, McElrath MJ, Gurunathan S, Ferrari G, Tomaras GD, Montefiori DC, Allen M, Chiu YL, Spearman P, Fuchs JD *et al INFECT DIS* 1992 7 1249-1259

745500 Effects of cytotoxic T lymphocytes (CTL) directed against a single simian immunodeficiency virus (SIV) Gag CTL epitope on the course of SIV_{mac239} infection. Allen TM, Jing P, Calore B, Horton H, O'Connor DH, Hanke T, Piekarczyk M, Ruddersdorf R, Mothe BR, Emerson C, Wilson N *et al J VIROL* 2002 76 20 10507-10511

745506 Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, Montefiori DC, Xu Y, Herndon JG, Wyatt LS, Candido MA *et al SCIENCE* 2001 292 5514 69-74

745508 Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-defective recombinant adenovirus vector. Calanzano AT, Koup RA, Roederer M, Baillet RT, Enama ME, Moodie Z, Gu L, Martin JE, Novik L, Chakrabarti BK, Butman BT *et al J INFECT DIS* 2006 194 12 1638-1649

- 745509 Efficacy of DNA and fowlpoxvirus prime/boost vaccines for simian/human immunodeficiency virus. Dale CJ, De Rose R, Stratov I, Chea S, Montefiori DC, Thomson S, Ramshaw IA, Coupar BE, Boyle DB, Law M, Kent SJ *J VIROL* 2004 78 24 13819-13828
- 745669 A shot in the arm for AIDS vaccine research. Ho DD *PLOS MED* 2005 2 2 e36
- 745754 Induction of long-lasting multi-specific CD8+ T cells by a four-component DNA-MVA/HIVA-RENTA candidate HIV-1 vaccine in rhesus macaques. Im EJ, Nkolola JP, di Gleria K, McMichael AJ, Hanke T *EUR J IMMUNOL* 2006 36 10 2574-2584
- 745760 A randomized, placebo-controlled phase I trial of DNA prime, recombinant fowlpox virus boost prophylactic vaccine for HIV-1. Kelleher AD, Puls RL, Bebbington M, Boyle D, French R, Kent SJ, Klippax S, Purcell DF, Thomson S, Wand H, Cooper DA *et al AIDS* 2006 20 2 294-297
- 745800 The rationale behind a vaccine based on multiple HIV antigens. Rollman E, Brave A, Boberg A, Gudmundsdottir L, Engstrom G, Isaguliantis M, Ljungberg K, Lundgren B, Blomberg P, Hinkula J, Hejdeman B *et al MICROBES INFECT* 2005 7 14 1414-1423
- 746295 Recent advances in the development of HIV-1 vaccines using replication-incompetent adenovirus vectors. Shiver JW, Emini EA *ANNU REV MED* 2004 55 355-372
- 746420 Multiprotein HIV type 1 clade B DNA/MVA vaccine: Construction, safety, and immunogenicity in macaques. Smith JM, Amara RR, McClure HM, Patel M, Sharma S, Yi H, Chennareddi L, Herndon JG, Butera ST, Heneine W, Ellenberger DL *et al AIDS RES HUM RETROVIRUSES* 2004 20 6 654-665
- 746507 Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 DNA candidate vaccine. Graham BS, Koup RA, Roederer M, Bailer RT, Enama ME, Moodie Z, Martin JE, McCluskey MM, Chakrabarti BK, Lamoreaux L, Andrews CA *et al J INFECT DIS* 2006 194 12 1650-1660
- 746509 Enhancement of MHC class I-restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination regime. Hanke T, Blanchard T, Schneider J, Hannan CM, Becker M, Gilbert SC, Hill AV, Smith GL, McMichael A *VACCINE* 1998 16 5 439-445
- 746745 Dose-response relationship of DNA and recombinant fowlpoxvirus prime-boost vaccines: Implications for future trials. De Rose R, Sullivan MT, Dale CJ, Kelleher AD, Emery S, Cooper DA, Ramshaw IA, Boyle DB, Kent SJ *HUM VACCIN* 2006 2 3 134-136
- 747372 AIDS: From crisis management to sustained strategic response. Piot P *LANCET* 2006 368 9534 526-530
- 747374 Progress and obstacles in the development of an AIDS vaccine. Letvin NL *NAT REV IMMUNOL* 2006 6 12 930-939
- 749956 Priming with a candidate HIV-1 clade A DNA vaccine followed by booster with HIV-1 clade A MVA vaccine in volunteers at low risk of HIV infection. Guimaraes-Walker A, Mackie N, McMichael A, Weber J, McCormack S, Ceberre I, Kay R, Hanke T, Dally L, Gibianski L, Legg K *et al AIDS VACCINE* 2004 3 Abs 55
- 750077 Generation of enhanced immune responses by consecutive immunisation with DNA and recombinant fowlpox viruses. Leong KH, Ramsay AJ, Morin MJ, Robinson HL, Boyle DB, Ramshaw IA. In: *VACCINES 95*, Brown F, Chanock H & Norrby E (Eds), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA 1995 327-331
- 750170 Predicting the impact of a nonsterilizing vaccine against human immunodeficiency virus. Davenport MP, Ribeiro RM, Chao DL, Perelson AS *J VIROL* 2004 78 20 11340-11351
- 750183 Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Daiesandro M, Scallan B, Ghayee J *et al Science* 1999 283 5403 857-860
- 751958 Host range restricted, non-replicating vaccinia virus vectors as vaccine candidates. Moss B, Carroll MW, Wyatt LS, Bennink JR, Hirsch VM, Goldstein S, Elkins WR, Fuerst TR, Lifson JD, Piatak M, Restifo NP *ADV EXP MED BIOL* 1996 397 7-13
- 751961 Nonreplicating vaccinia vector efficiently expresses recombinant genes. Sulter G, Moss B *PROC NATL ACAD SCI USA* 1992 89 22 10847-10851
- 753120 2004 Mid-year progress report. International AIDS Vaccine Initiative *COMPANY PUBLICATION* 2004 November 15 <http://www.iavi.org/>
- 753139 Safety and immunogenicity of DNA and MVA HIVA vaccines in phase I HIV-1 vaccine trials in Nairobi, Kenya. Jaoko W, Omosa G, Bhatt K, Matu L, Ogutu H, Wakasiaka S, Odada J, Anzala O, Bashir F, Oyaro M, Indangasi J *et al AIDS VACCINE* 2004 3 Abs 56
- 754700 Clinical experience with plasmid DNA- and modified vaccinia virus Ankara-vectored human immunodeficiency virus type 1 clade A vaccine focusing on T-cell induction. Hanke T, McMichael AJ, Dorrell L *J GEN VIROL* 2007 88 1 1-12
- 754704 International AIDS Vaccine Initiative abandons lead vaccine candidate, citing poor responses. Meldrum J *PRESS RELEASE* 2004 September 01 <http://www.aidsmap.com>