Drug evaluation: DNA/MVA prime-boost HIV vaccine
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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 (pTHr 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

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)	

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 IFNγ 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 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 pTH.HM 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 pTH.HIVA, 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 (10^7 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 (5×10^5 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 (5×10^8 pfu) – both the GMP-grade constructs – showed distinct T-cellular immunity, as detected by a validated IFNγ 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 5×10^7 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 IFNγ+CD69+CD8+) after vaccination, and detectable, but low specific cellular

immunity (50 to 150 IFNγ spot-forming units (sfu)/10^6 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 RT 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 (10^6 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 1 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 (2×10^6 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 IFNγ 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 μg) 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* IFNγ 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* IFNγ 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/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* IFNγ 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 grant 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
In vivo	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 *ex vivo* ELISpot assays	720674
In vivo	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 (5×10^8 pfu intradermally). with subsequent SIV$_{mac251}$ challenge.	ELISpot assays showed potent immune responses to the vaccines	720664
In vivo	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 *in vitro* peptide restimulation.	720658
In vivo	Immunogenicity	Five macaques administered DNA prime (1 mg intramuscularly) and MVA boost (10^6 pfu) with both HIVA and RENTA immunogens.	Low *ex vivo* 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 IFNγ 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 IFNγ 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 10^8 pfu) or placebo in 24 healthy HIV-negative individuals (eight received vaccine and four received placebo in each group)	Ex *vivo* 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

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