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Safety and immunogenicity of recombinant low-dosage HIV-1 A vaccine candidates vectored by plasmid pTHr DNA or modified vaccinia virus Ankara (MVA) in humans in East Africa☆

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Summary: The safety and immunogenicity of plasmid pTHr DNA, modified vaccinia virus Ankara (MVA) human immunodeficiency virus type 1 (HIV-1) vaccine candidates were evaluated in four Phase I clinical trials in Kenya and Uganda. Both vaccines, expressing HIV-1 subtype A gag p24/p17 and a string of CD8 T-cell epitopes (HIVA), were generally safe and well-tolerated. At the dosage levels and intervals tested, the percentage of vaccine recipients with HIV-1-specific cell-mediated immune responses, assessed by a validated *ex vivo* interferon gamma (IFN-γ) ELISPOT assay and Cytokine Flow Cytometry (CFC), did not significantly differ from placebo recipients. These trials demonstrated the feasibility of conducting high-quality Phase 1 trials in Africa.

Introduction

The development of a safe and efficacious preventive HIV vaccine remains a global priority. Hence it is important to design HIV vaccines for, and to test them in Africa, where HIV prevention is critical. Two HIV vaccine candidates were developed in a partnership between the University of Nairobi's Kenya AIDS Vaccine Initiative (KAVI), the Medical Research Council, University of Oxford and the International AIDS Vaccine Initiative (IAVI). A plasmid DNA (pTHr) and a modified vaccinia virus Ankara (MVA) expressed HIV-1 clade A gag p17/p24 fused to a string of CD8+ T-cell epitopes (HIVA) [1]. These epitopes, derived from HIV-1 gag, pol, nef and env, were immunodominant in HIV-1-infected persons and presented by HLA alleles common in Kenya [1,2]. In addition, the HIV gene insert coded for epitopes of HIV-1 env [3] and SIV gag [4] recognized by mouse and *Rhesus* CD8+ T cells, respectively. The two vaccine candidates, pTHr.HIVA DNA and MVA.HIVA, were safe in routine pre-clinical testing [5] and induced CD8+ T-cell responses in mice [6] and macaques [7]. In their first human clinical trials, conducted at the University of Oxford, UK, both vaccines were well tolerated and preliminary immunogenicity findings warranted further clinical evaluation [8,9]. In East Africa, where HIV-1 subtype A is predominant [10], four Phase 1 clinical trials were conducted to evaluate the safety and the immunogenicity of the vaccine candidates, either given alone or in a ''prime—boost'' combination. Along with one pioneering trial in Uganda [11], these studies represent the first preventive HIV vaccine clinical trials conducted in Africa; they demonstrated the feasibility of conducting high-quality HIV vaccine clinical research in Africa.

Materials and methods

Study vaccines

The vaccine candidates have been described previously [1,5—8]. The pTHr.HIVA DNA vaccine and placebo (saline in IAVI 002) and Tris—EDTA—saline (10 mM Tris—HCl buffer, 1 mM EDTA and 0.9% saline, pH 7.1 in IAVI 009) were manufactured by Cobra Bio-Manufacturing (Keele, UK). The MVA.HIVA vaccine and placebo (Tris-buffered saline, 10 mM Tris—HCl buffer, 140 mM NaCl, pH 7.7) were manufactured by Impfstoffwerke Dessau-Thornau GmbH (Rosslau, Germany). pTHr.HIVA DNA was administered intramuscularly at 0.5 mg in 1.0 mL per dose and MVA.HIVA intradermally at 5×10^7 plaque-forming units (pfu) in 0.1 mL per dose.

Regulatory and ethics approval

The clinical trial protocols and related documents were approved by the respective regulatory authorities and independent ethics committees. Trial data were reviewed by independent Data Monitoring and Ethics Committees (DMEC) with national and international members. The studies were conducted in accordance with International Conference on Harmonization — Good Clinical Practice (ICH-GCP) guidelines. All volunteers provided written informed consent before any trial related procedures were performed.

Study design and vaccination regimens

IAVI 002, IAVI 004 and IAVI 008

Table 1 summarizes the different study designs. Three small Phase 1 trials were conducted at KAVI in Nairobi. The first two trials were randomized, double-blind, placebo-controlled trials enrolling 18 volunteers each. In IAVI 002, 2 doses of pTHr.HIVA DNA vaccine or placebo were given 21 days apart. In IAVI 004, 2 doses of MVA.HIVA or placebo were given 28 days apart. In IAVI 008 (Rollover trial), 10 volunteers who had previously participated in IAVI 002, consented to receive 2 doses of MVA.HIVA as boost, 28 days apart, at least 32 weeks after their first vaccinations and were followed up for an additional 18 months. Eleven volunteers from the IAVI 004 trial consented to receive a third dose of MVA.HIVA or placebo after the 18 months follow-up period, and were followed up for a further three months.

IAVI 009

This double-blind, randomized, placebo-controlled trial was conducted at the Uganda Virus Research Institute (UVRI)—IAVI Vaccine Trial Unit (VTU) in Entebbe and enrolled 50 healthy volunteers (Table 1). Group A ($n = 20$) was primed with one dose of pTHr.HIVA DNA followed by one dose of DNA placebo, and Group B ($n = 20$) was primed with two doses of pTHr.HIVA DNA. Volunteers in both groups were boosted with two doses of MVA.HIVA. Ten participants (Group C) received 4 injections of placebo (Table 1). All volunteers were followed up for 18 months.

Volunteer recruitment and study population

Eligible volunteers between 18 and 60 (IAVI 002, 004, 008) or 18 and 50 (IAVI 009) years of age were recruited through advertisements and information seminars held in different communities and at the clinical study sites. Eligible volunteers were free from significant chronic illnesses, had

Table 1 Study designs

Study		*N*	Weeks					*N*	Weeks		*N*	Week
			Wk 0	Wk 3	Wk 4	Wk 20	Wk 30		≥Wk 35	+4 Wks		≥Wk 78
002/008	A	13	DNA[a]	DNA				7	MVA[b]	MVA		
KAVI	B	5	Placebo	Placebo				3	MVA	MVA		
004	A	12	MVA		MVA						9	MVA
KAVI	B	6	Placebo		Placebo						2	Placebo
009	A	20	DNA		Placebo	MVA	MVA					
UVRI	B	20	DNA		DNA	MVA	MVA					
	C	10	Placebo		Placebo	Placebo	Placebo					

[a] 0.5 mg in 1.0 mL per dose given intramuscularly into the deltoid region.
[b] 5×10^7 pfu in 0.1 mL per dose given intradermally over the deltoid region.

a normal physical exam; routine biochemistry and haematology laboratory parameters within normal ranges; were seronegative for HIV-1 and HIV-2 and at low risk of HIV-infection; negative for Hepatitis B surface antigen, syphilis and antibodies against Hepatitis C virus; and for anti-nuclear antibodies (ANA) or anti-double stranded DNA. Women of childbearing age were neither pregnant nor breastfeeding, willing to use an approved method of contraception and not planning a pregnancy in the 4 months after the last injection. There was no exclusion or stratification of participants based on pre-existing immunity to vaccinia.

Safety assessment

Local reactogenicity events (pain, tenderness, erythema/discoloration, and induration) and systemic events (fever, headache, malaise, myalgia, and nausea) were recorded prospectively up to 14 days after each vaccination in IAVI 002, 004 and 008, and up to 10 days after each vaccination in IAVI 009. Unsolicited adverse events were collected throughout the study by interim medical history, physical examination, and clinical safety laboratory tests. All adverse events were graded for severity according to pre-defined criteria and, with the exception of the solicited reactogenicity events assessed as described above, were assessed for relationship to study vaccines. Serious adverse events (SAEs) were defined according to ICH/GCP guidelines. In IAVI 002 and 004, clinical and laboratory abnormalities of grade 3 and 4 were also reported as SAEs, in IAVI 008 and 009 such events were reported expeditiously, but not defined as SAEs unless they met the ICH-GCP criteria for serious.

Routine safety parameters were tested at the respective clinical laboratories at KAVI and UVRI. Quality was assured through internal and external quality control/quality assurance programs. Standard operating procedures (SOPs) were developed and all clinic and laboratory staff was trained in SOPs, ICH-GCP and/or Good Clinical and Laboratory Practices (GCLP).

Immunogenicity assays

Laboratory SOPs including an SOP for PBMC processing were developed and standardized across all sites. All staff was adequately trained. All samples were logged into an electronic system called LIMS (Laboratory Information Management Software) for tracking purposes.

Cellular immunogenicity

Immunogenicity assays were performed on site and all blood samples were processed within 4 h of blood draw. ELISPOT assays were performed on freshly isolated peripheral blood mononuclear cells (PBMCs) to detect T cells releasing interferon-gamma (IFN-γ) upon specific peptide stimulus. The IAVI Core Clinical Immunology Laboratory at Imperial College London provided supervision, quality control and standardized reagents for the immunogenicity assays. HPLC purified (>90%) synthetic peptides (Anaspec, US) covering the HIVA gene inserts (15-mers overlapping by 11 amino acids) were used in 4 complementary pools of 22—23 peptides or one large pool of 90 gag peptides, and a pool of 23 peptides to cover the CTL epitope string. Responses were considered positive based upon pre-set criteria determined through assay qualification at the IAVI Core Laboratory and on site. Panels of unvaccinated, HIV-negative PBMC were tested in the ELISPOT assay in order to get a distribution of data from known negative samples, and the cut-off for an assay positive was determined at 99%/98% confidence that the test sample was not negative. Assay qualification included assessment of reproducibility (intra-assay, inter-assay, inter-operator) for each site. As well as internal assay QC, external quality assurance testing included proficiency testing using a panel of blinded samples and antigens sent by the IAVI core lab. Independent testing of frozen samples from each site at the IAVI core laboratory and on site audit demonstrated that the assay performance was consistent throughout the duration of the study and independent of site or operator.

Positive (FEC, EBV-Flu-CMV, SEB, PHA) and negative (PBMCs with no peptide stimulation) controls were included in all assays. The definition of a positive response was as follows: (1) >39 SFU/10^6 cells above background if one peptide pool scored positive at a single time point; or >30 SFU/10^6 cells above background if multiple peptide pools scored positive at a single time point, (2) >4× mean background, (3) background < 55 SFU/10^6 cells and (4) coefficient of variation across the replicate wells <70%. A subset of samples was further assessed by a cytokine flow cytometry assay measuring IFN-γ (IFN-γ CFC, Fastimmune, CD4, CD69, IFN-γ plus CD3, Becton Dickinson). This was a non-validated research

assay performed on site. A sample was defined positive if the CD3+/CD69+/CD4+/IFN-γ+ gated population was >0.05% (CD4) and the CD3+/CD69+/CD4−/IFN-γ+ was >0.20% (CD8). The total number of gated events had to be >15,000 and Mock % gated had to be <0.5%.

Humoral immunogenicity

Standard HIV testing by commercially available ELISA kits was performed throughout the study to detect any HIV specific vaccine-induced antibodies. To identify intercurrent HIV-infections, a specific HIV testing algorithm including serological assays and PCR was developed.

Anti-MVA immunity

Testing for pre-existing anti-vaccinia or specific MVA vector induced humoral or cellular immunity was not performed.

Data management and analysis

Data were collected in real time using standard data collection forms and entered into an Internet-based data entry system (IDES, The EMMES Corporation, Rockville, MD, USA). To assess vaccine safety, the rates of adverse events among vaccine recipients and placebo recipients were compared using Fisher's exact one-tailed test. Maximum local reactions and maximum systemic events are presented by severity and proportion of volunteers experiencing these events. Unsolicited adverse events are described by severity and relationship to study vaccines.

Results

Table 2 gives the age and gender distribution of volunteers for each study. Mean age across all trials was 25 and 29 years for men and women, respectively. No volunteer withdrew from the trials due to a vaccine-related adverse event, and 92% of volunteers completed the studies.

Safety

Local reactogenicity

pTHr.HIVA DNA. In IAVI 002, no local reactogenicity was observed following pTHr.HIVA DNA. In IAVI 009, local reactogenicity was observed in 12.5% and 5.0% of volunteers after the first and second dose of pTHr.HIVA DNA, respectively. These rates were not significantly different from the placebo group (0% and 10.7%, respectively). All local reactions were mild pain or tenderness after pTHr.HIVA DNA and mild induration after placebo (data not shown).

MVA.HIVA. In IAVI 004 and IAVI 008, 91% of vaccine recipients had moderate (19/22) or severe (1/22) reactions after the first or second MVA.HIVA injection versus 0% of placebo recipients ($p < 0.0001$). Induration at the vaccination site occurred in all 22 vaccine recipients (31.8% mild, 63.6% moderate, 4.5% severe — defined as >25 mm in diameter) and all 6 placebo recipients (100% mild). Seventeen vaccine recipients (77.3%) also had some skin discoloration at the vaccination site after the first ($n = 12$) and/or second ($n = 14$) and/or third ($n = 4$) vaccination; discoloration was mild except in three volunteers, in whom it was moderate. In a few vaccine recipients some discoloration persisted to the end of the study. No discoloration was observed in the placebo group.

In IAVI 009, following MVA.HIVA vaccination, 83% and 100% of recipients experienced local reactogenicity events after the first and second injections, respectively, compared to 44% and 57% of volunteers given the corresponding intradermal placebo injection. The difference was statistically significant after both the first ($p = 0.0275$) and the second injection ($p = 0.0033$). The overall maximum local reaction after any MVA.HIVA or placebo vaccination is shown in Fig. 1. There were no severe reactions. The most common local reactions of moderate severity after MVA.HIVA were induration in 53% and discoloration in 28%. In general, both induration and discoloration persisted for up to 7—10 days post-MVA.HIVA vaccination, and for less than 2 days post-placebo.

Systemic events

pTHr.HIVA DNA. In IAVI 002 and 009, only mild events were observed following pTHr.HIVA DNA. In IAVI 002, mild nausea was reported by one participant, after the first vaccination. In IAVI 009, mild fever, headache, and/or myalgia were observed in 5% (2/40) and 15% (3/20) vaccine recipients after the first and second pTHr.HIVA DNA injections, respectively, compared with 0/10 and 2/10 placebo recipients (one headache, one malaise + nausea).

MVA.HIVA. Systemic symptoms were recorded more frequently for the MVA.HIVA vaccine than for pTHr.HIVA DNA, but reactions were generally mild (Fig. 1). In IAVI 004, 4 (33%) recipients reported mild nausea, subjective fever, headache and/or malaise. In IAVI 008, 4 (40%) volunteers reported mild subjective fever, headache, myalgia, nausea and/or malaise, while one (10%) volunteer reported moderate headache, mild malaise and mild nausea. The duration of any event was no more than 1 or 2 days. No

Table 2 Demographic characteristics of trial volunteers

		Nairobi (KAVI)						Uganda (UVRI)	
		002, $N = 18$		004, $N = 18$		008 Rollover from 002, $N = 10$		009, $N = 50$	
		Male	Female	Male	Female	Male	Female	Male	Female
# Volunteers		15	3	16	2	8	2	42	8
Age (years)	Mean	26.0	26.0	27.2	32.0	23.9	23.5	23.9	28.9
	Min—Max	19—41	21—31	19—31	26—38	19—31	21—26	18—33	20—40

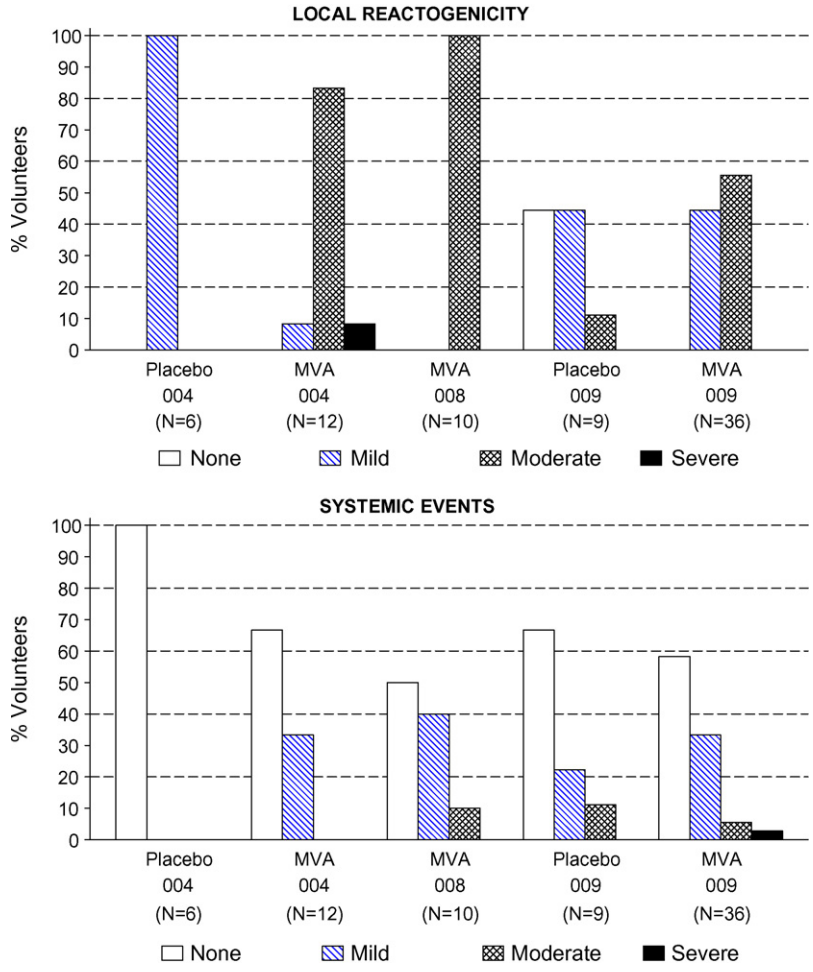

Figure 1 Maximum severity of local reactogenicity and systemic events after any MVA.HIVA or placebo vaccination by study.

systemic events were reported in IAVI 004 after the MVA.HIVA placebo.

In IAVI 009, the rate of systemic events in MVA.HIVA vaccine recipients (15/36) did not differ from placebo recipients (3/9), i.e., 42% versus 33% ($p = 0.4763$) One participant had severe myalgia one week after the second MVA.HIVA vaccination.

Adverse events

Volunteers from IAVI 002, 004 and 008 reported 87 adverse events within 21 days post-vaccination: 21 (24%) following pTHr.HIVA DNA, 60 (69%) following MVA.HIVA, and 6 (7%) following placebo administration. Overall, 7 (8%) were moderate or severe, and 23 (26%) were considered possibly, probably or definitely vaccine related. Definitely related adverse events were mainly local reactions after MVA.HIVA, including skin discoloration and induration, which persisted beyond the period of immediate post-vaccination follow-up. Five participants had lymphadenopathy judged to be related to the vaccine candidate. Three adverse events of moderate or greater severity were assessed as possibly, probably, or definitely related to vaccine candidates: two (moderate skin peeling and neutropenia) after MVA.HIVA injection and one (grade 3 elevated total bilirubin) after placebo.

Volunteers in IAVI 009 reported 99 adverse events within 28 days post-vaccination, of which 43, 27 and 29 were after pTHr.HIVA DNA, MVA.HIVA, and placebo, respectively. Overall, 71% were graded moderate, 6% severe, and 2% very severe. All were judged to be unrelated to study vaccine, except two possibly related events: one mild tinnitus after the fourth placebo injection and one mild viral upper respiratory tract infection after the first MVA.HIVA injection. One study participant acquired HIV-infection one year after having received a single dose of 0.5 mg pTHr.HIVA DNA. Further vaccinations in this volunteer had previously been discontinued due to increase in severity of pre-existing hyperbilirubinemia. This volunteer's immunogenicity data were censored from the analysis.

Electrocardiograms and cardiac enzymes were not prospectively performed. No cardiac events suggestive of pericarditis or myocarditis were reported in these studies.

Serious adverse events. There were 16 serious adverse events (including 4 severe or very severe laboratory abnormalities) reported during the trials, but none was judged to be related to either study vaccine.

Laboratory results. Most abnormalities were isolated, had no clinical correlate, resolved spontaneously, and were judged unrelated to study vaccines. In IAVI 002 one pTHr.HIVA DNA recipient had grade 3 (>6× Upper Limit

Normal) elevated Aspartate Aminotransferase (AST), which was attributed to malaria confirmed by blood smear. In IAVI 004 one placebo recipient, who had normal total and direct bilirubin levels at baseline, developed persistently elevated bilirubin levels with no other pathology. One MVA.HIVA vaccine recipient had severe thrombocytopenia without a clinical correlate, which resolved spontaneously over time. In IAVI 009, the most commonly observed laboratory abnormalities were neutropenia, thrombocytopenia and hyperbilirubinemia. In a clinically asymptomatic volunteer, very severe neutropenia was noted in a blood specimen taken immediately prior to the second vaccination with pTHr.HIVA. It resolved spontaneously. Highly fluctuating total bilirubin levels were observed in three otherwise healthy, asymptomatic volunteers, suggesting Gilbert syndrome, a congenital condition.

No antibodies to double-stranded DNA were detected in any of the volunteers. There was one pregnancy in a placebo recipient in IAVI 004.

Immunogenicity

Vaccine-induced T-cell responses

In IAVI 002, the number of vaccine-induced HIV-1-specific T-cell responses detectable in *ex vivo* IFN-γ ELISPOT assay in at least one peptide pool on at least one occasion was 2/13 (15.4%). In IAVI 004 there were 4/12 (33.3%) MVA.HIVA responders and 2/6 (33.3%) placebo responders. Of the 10 MVA.HIVA recipients in IAVI 008 there was 1 responder (10%) following the first dose of MVA.HIVA. This volunteer had been primed with 2 doses of pTHr.HIVA DNA 2 years previously. The two placebo responses in IAVI 004 were likely due to variation in background levels. There was no significant difference in percentage of responders between pTHr.HIVA DNA (15.4%) and placebo (18.2%) recipients ($p = 0.7671$ by Fisher's exact one-tailed test), or between MVA.HIVA (22.7%) and placebo (18.2%) recipients ($p = 0.5711$).

In IAVI 009, 13.2% (5/38) of volunteers had a detectable response in the *ex vivo* IFN-γ ELISPOT assay after receiving MVA.HIVA booster injections. Three of these volunteers had responses at a single time point, and 2 had responses present across multiple time points. No responses were observed in placebo recipients, or prior to the MVA.HIVA boost. The number of DNA priming doses (1 versus 2) had no effect on the response (Group A: 3/18; Group B: 2/20), and the difference in responses between vaccine (5/38) and placebo (0/9) recipients did not reach statistical significance ($p = 0.5438$).

IFN-γ CFC was used to further characterize responses as CD4+ or CD8+. As predicted by the relatively modest magnitude of the ELISPOT responses, there were only 9 positive assays in 3 volunteers (1 placebo recipient, 2 vaccine recipients) in IAVI 004. Three of the nine samples were also positive by IFN-γ ELISPOT (2 samples from the placebo recipient, 1 from a vaccine recipient). 2/3 responders had a single CD4 response and multiple CD8 responses. The third responder had no CD4 response and a CD8 response at every visit but one, including baseline.

In IAVI 009 ten volunteers each had a positive IFN-γ CFC response at a single time point only: 1 (10.0%) post-placebo, 3 (7.5%) post-DNA, and 6 (15.8%) post-MVA boost. Only one of the ten samples was also positive by IFN-γ ELISPOT, 4 weeks after receiving the first DNA vaccination. All had a CD4 response and only 3 had a CD8 response.

Vaccine-induced antibody responses

There were no positive HIV-test results due to vaccine-induced antibodies at any time during the study and at final study visit by standard HIV ELISA tests.

Discussion

Overall, the pTHr.HIVA DNA and MVA.HIVA vaccine candidates appear to be safe and generally well-tolerated. These findings corroborate the safety results of clinical trials conducted in the UK testing the same vaccines at the same dosage levels and using the same delivery route [8,9] and the safety results of larger multicenter studies conducted in both Europe and Africa, using higher dosage levels of MVA.HIVA and other routes of administration (intramuscularly, subcutaneously) [13]. The high frequency of local reactogenicity following MVA.HIVA injection could be partly explained by the intradermal route of administration, since overall more than half the placebo recipients also had mild or moderate local reactions.

The definition of prospectively collected local reactogenicity events, their grading and the time period of immediate post-vaccination follow-up were the similar for all studies. No diary cards were used. All aspects of collecting local reactogenicity data were the same at both sites. There are no obvious reasons to explain the observed differences in frequencies of local reactogenicity events by site.

The frequency of unsolicited adverse events reported was not statistically significant different between vaccine and placebo recipients. There was no pattern of laboratory abnormalities that raised a safety concern. Preliminary results from a study conducted in several African countries including Kenya and Uganda to establish local laboratory reference ranges in clinically healthy HIV-seronegative individuals suggest that the local reference ranges of some safety parameters overlap with severity grades 1 and above as defined by the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (publish date: December 2004, http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/Safety/DAIDSAEGradingTable.pdf). Most of the laboratory abnormalities observed in the studies described here would not be classified as abnormal if local reference ranges were applied (AIDS Vaccine 2007, Seattle, IAVI Satellite Meeting). No cardiac events were noted in these studies. This is in line with results from studies with other recombinant, non-replicating, non-HIV MVA-based vector vaccines [14].

No antibodies against double-stranded DNA were detected in any volunteers, which may alleviate the original concern about the induction of autoimmunity following DNA vaccine administration [15].

The immunogenicity of the two vaccine candidates given either alone or in a prime—boost regimen was poor. Although some responses were observed, there was no statistically significant difference in percentage of responders between vaccine and placebo recipients in any of these small trials. There was no correlation between positive ELISPOT

responses and moderate or greater local reactogenicity or systemic events (data not shown).

Another small trial, however, testing the highest doses of pTHr.HIVA DNA, a shorter interval between DNA prime and MVA boost, and a shorter interval between boost and immunogenicity testing showed improved immunogenicity by ELISPOT assay. In this study CFC had a lower sensitivity compared to the ELISPOT assay [12]. This has also been our experience in subsequent clinical studies. The CFC assay at the time when we were conducting the trials described here was not as well qualified as ELISPOT. Lack of correlation between CFC and ELISPOT might be attributable to working at the limit of detection of the assays due to poor vaccine-induced immune response.

The DNA.HIVA and MVA.HIVA vaccines were designed to elicit T-cell responses [9]. They did not appear to induce HIV gag specific antibodies, which is in line with data from earlier small Phase I studies conducted in the UK [9].

Naked DNA vaccines have a number of advantages over conventional vaccines and were shown to elicit cellular immune responses in mice and macaques. However, immune responses induced by HIV DNA vaccines in human trials have been generally weak. Therefore, approaches to improve the potency of DNA vaccines in the clinic are of great importance [16]. Various strategies to enhance immunogenicity and T-cell priming of DNA vaccines by improved expression and/or enhanced delivery have been investigated [17—23]. Better immune responses have been observed when DNA vaccines are given using biojector [17] or gene-gun [18], in prime—boost regimens with plasmid DNA encoding for GM-CSF [19], and with adjuvants [20,21].

The low immune response induced by MVA.HIVA contrasts sharply with the results observed in the initial study of MVA.HIVA, done in a predominantly Caucasian population in the UK [24]. Two small, subsequent trials, one in HIV-negative [12] and another in HIV-positive [25] volunteers used more sensitive immunogenicity assays. In those studies, the pTHr.HIVA DNA vaccine alone primed consistently but weakly and mainly CD4+ T-cell responses below the detection threshold of the *ex vivo* IFN-γ ELISPOT assay, while the MVA.HIVA vaccine consistently boosted both CD4+ and CD8+ T cell reponses, which were particularly strong if the HIV-1-specific T cells were primed efficiently, e.g., by HIV-1 infection [25].

The findings described here are product-specific, and cannot be applied to other HIV vaccine candidates using DNA- or MVA-based vaccines. Potential differences between these and other similar vaccine constructs are many fold and relate to the construction itself, production cell lines, sources and types of inserts, codon-optimization, genetic stability, route of administration and other factors. Subsequently IAVI conducted clinical studies with other MVA HIV vaccine constructs that showed much better immunogenicity by IFN-γ ELISPOT than MVA.HIVA [26,27]. These constructs differ from the MVA.HIVA in deletion sites, plaque purification process, insertion sites, selection of genes, route of administration and other factors.

Conclusions

The clinical trials reported here investigated the safety, tolerability and immunogenicity of two HIV-1 candidate vaccines, a plasmid DNA vaccine and a vector-based vaccine, in healthy adult African volunteers. These studies represent some of the first HIV vaccine clinical trials to be performed in sub-Saharan Africa. As such, they are important in demonstrating the feasibility of conducting high-quality HIV vaccine research in this region and contributed to capacity building and establishment of clinical trial infrastructure in East Africa.

The trials demonstrate the overall safety of both DNA- and MVA-based vaccines in these populations. Only limited data still exist at present regarding the safety of candidate MVA-based HIV vaccines in prime—boost regimens, while several are entering or approaching clinical trials throughout the world. The immunogenicity of the two vaccine candidates, as evaluated by the respective study designs and immunological assays performed, was disappointing. Although the clinical development of the two vaccine candidates tested is currently not pursued for prevention of HIV/AIDS, the studies provided some of the first data on the safety of MVA vaccines.

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References

[1] Hanke T, McMichael A. Design and construction of an experimental HIV-I vaccine for a year-2000 clinical trial in Kenya. Nat Med 2000;6(9):951—5.

[2] Dorrell L, Dong T, Ogg GS, Lister S, McAdam S, Rostron T, et al. Distinct recognition of clade A HIV-1 epitopes by cytotoxic T lymphocytes generated from donors infected in Africa. J Virol 1999;73(2):1708—14.

[3] Takahashi H, Houghten R, Putney SD, Margulies DH, Moss B, Germain RN, et al. Structural requirements for class I MHC molecule-mediated antigen presentation and cytotoxic T cell recognition of immunodominant determinant of the human immunodeficiency virus envelope protein. J Exp Med 1989;170:2023—35.

[4] Allen TM, Sidney J, del Guercio MF, Glickman RL, Lensmeyer GL, Wiebe DA, et al. Characterization of the peptide-binding motif of a rhesus MHC class I molecule (Mamu-A*01) that binds an immunodominant CTL epitope from SIV. J Immunol 1998;160:6062—71.

[5] Hanke T, McMichael AJ, Samuel RV, et al. 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. Vaccine 2002;21:108—14.

[6] Hanke T, Blanchard TJ, Schneider J, et al. Immunogenicities of intravenous and intramuscular administrations of modified vaccinia virus Ankara-based multi-CTL epitope vaccine for human immunodeficiency virus type 1 in mice. J Gen Virol 1998;79(Pt 1):83—90.

[7] Wee EG, Patel S, McMichael AJ, Hanke T. A DNA/MVA-based candidate human immunodeficiency virus vaccine for Kenya induces multi-specific T cell responses in *Rhesus Macaques*. J Gen Virol 2002;83(Pt 1):75—80.

[8] Cebere I, Dorrell L, McShane H, Simmons A, McCormack S, Schmidt C, et al. 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. Vaccine 2006;24(4):417—25.

[9] Hanke T, McMichael AJ, Dorell L. Clinical experience with plasmid DNA- and modified vaccinia virus Ankara-vectored human immunodeficiency virus type 1 clade A vaccine focusing on T-cell induction. J Gen Virol 2007;88:1—12.

[10] Lihana RW, Khamadi SA, Kiptoo MK, Kinyua JG, Lagat N, Magoma GN, et al. HIV type 1 subtypes among STI patients in Nairobi: a genotypic study based on partial pol gene sequencing. AIDS Res Hum Retroviruses 2006;22(11):1172—7.

[11] Mugerwa RD, Kaleebu P, Mugyenyi P, Katongole-Mbidde E, Hom DL, Byaruhanga R, et al. First trial of the HIV-1 vaccine in Africa: Ugandan experience. BMJ 2002;324(7331):226—9.

[12] Goonetilleke N, Moore S, Dally L, Winstone N, Cebere I, Mahmoud A, et al. Induction of multi-functional HIV-1-specific T cells capable of proliferation in healthy subjects by a prime—boost regimen of DNA- and MVA-vectored vaccines expressing HIV-1 gag coupled to CD8+ T cell epitopes. J Virol 2006;80(10):4717—28.

[13] Peters BS, Jaoko W, Vardas E, Panayotakopoulos G, Fast P, Schmidt C, et al. Studies of a prophylactic HIV-1 vaccine candidate based on modified vaccinia virus Ankara (MVA) with and without DNA priming: effects of dosage and route on safety and immunogenicity. Vaccine 2007;25:2120—7.

[14] Parrino J, McCurdy LH, Larkin BD, Gordon IJ, Rucker SE, Enama ME, et al. Safety, immunogenicity and efficacy of modified vaccinia Ankara (MVA) against Dryvax® challenge in vaccinia-naïve and vaccinia-immune individuals. Vaccine 2007;25:1513—25.

[15] Robertson JS, Griffiths E. Assuring the quality, safety, and efficacy of DNA vaccines. Mol Biotechnol 2001;17:143—9.

[16] Giri M, Ugen KE, Weiner DB. DNA vaccines against human immunodeficiency virus type 1 in the past decade. Clin Microbiol Rev 2004;17(2):370—89.

[17] Sheets RL, Stein J, Manetz TS, Duffy C, Nason M, Andrews C, et al. Biodistribution of DNA plasmid vaccines against HIV-1, Ebola, Severe Acute Respiratory Syndrome, or West Nile virus is similar, without integration, despite differing plasmid backbones or gene inserts. Toxicol Sci 2006;91(2):610—9.

[18] Haynes JR, Fuller DH, McCabe D, Swain WF, Widera G. Induction and characterization of humoral and cellular immune responses elicited via gene gun-mediated nucleic acid immunization. Adv Drug Deliv Rev 1996;21:3—18.

[19] Sedegah M, Weiss W, Sacci Jr JB, Charoenvit Y, Hedstrom R, Gowda K, et al. Improving protective immunity induced by DNA-based immunization: priming with antigen and GM-CSF-encoding plasmid DNA and boosting with antigen-expressed recombinant poxvirus. J Immunol 2000;164(11):5905—12.

[20] Kim JJ, Yang JS, VanCott TC, Lee DJ, Manson KH, Wyand MS, et al. Modulation of antigen-specific humoral responses in *Rhesus Macaques* by using cytokine cDNAs as DNA vaccine adjuvants. J Virol 2000;74(7):3427—9.

[21] Calarota SA, Weiner DB. Enhancement of human immunodeficiency virus type 1-DNA vaccine potency through incorporation of T-helper 1 molecular adjuvants. Immunol Rev 2004;199:84—99.

[22] Heller LC, Ugen K, Heller R. Electroporation for targeted gene transfer. Expert Opin Drug Deliv 2005;2:255—68.

[23] Dean HJ. Epidermal delivery of protein and DNA vaccines. Expert Opin Drug Deliv 2005;2:227—36.

[24] Mwau M, Cebere I, Sutton J, Chikoti P, Winstone N, Wee EG, et al. A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trial: stimulation of HIV-1 specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. J Gen Virol 2004;85(Pt 4):911—9.

[25] Dorrell L, Yang H, Ondondo B, et al. Quantitative and qualitative changes in virus-specific CD8+ T cell responses in HIV-1-infected individuals on antiretroviral therapy induced by vaccination with recombinant modified virus Ankara (MVA) expressing HIV-1 gag. J Virol 2006;80:4705—16.

[26] Cellular and Humoral Immunogenicity of ADMVA, a Clade C/B' MVA-based HIV-1 Candidate Vaccine, in healthy volunteers: presentation at AIDS Vaccine 2007. Seattle, Washington, USA; August 20—23, 2007.

[27] Study to Evaluate the Safety and Immunogenicity of a Recombinant Modified Vaccinia: Ankara Virus (TBC-M4) multigenic HIV subtype C Vaccine. AIDS Vaccine 2007. Seattle, Washington, USA; August 20—23, 2007 [abstract P06-02].