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Broad and potent immune responses to a low dose intradermal HIV-1 DNA boosted with HIV-1 recombinant MVA among healthy adults in Tanzania☆,☆☆

Muhammad Bakari[a,*,1,2], Said Aboud[b,d,1,2], Charlotta Nilsson[d,e,2], Joel Francis[f,2], Deus Buma[h,2], Candida Moshiro[c,2], Eric A. Aris[g,2], Eligius F. Lyamuya[b,2], Mohamed Janabi[g,2], Karina Godoy-Ramirez[e,2], Agricola Joachim[b,d,2], Victoria R. Polonis[i,2], Andreas Bråve[e,2], Patricia Earl[j,2], Merlin Robb[k,2], Mary Marovich[i,2], Britta Wahren[d,e,2], Kisali Pallangyo[a,2], Gunnel Biberfeld[d,e,2], Fred Mhalu[b,2], Eric Sandström[l,2]

[a] Department of Internal Medicine, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania
[b] Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania
[c] Department of Epidemiology and Biostatistics, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania
[d] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden
[e] Swedish Institute for Communicable Disease Control (SMI), Solna, Sweden
[f] National Institute for Medical Research (NIMR), Dar es Salaam, Tanzania
[g] Department of Internal Medicine, Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania
[h] Department of Pharmacy, Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania
[i] Walter Reed Army Institute of Research (WRAIR), Rockville, MD, USA
[j] National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH), Bethesda, MD, USA
[k] The Henry M. Jackson Foundation, Rockville, MD, USA
[l] Venhälsan, Karolinska Institutet (KI), Södersjukhuset, Stockholm, Sweden

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ABSTRACT

Background: We conducted a phase I/II randomized placebo-controlled trial with the aim of exploring whether priming with a low intradermal dose of a multiclade, multigene HIV-1 DNA vaccine could improve the immunogenicity of the same vaccine given intramuscularly prior to boosting with a heterologous HIV-1 MVA among healthy adults in Dar es Salaam, Tanzania.
Methods: Sixty HIV-uninfected volunteers were randomized to receive DNA plasmid vaccine 1 mg intradermally (id), $n = 20$, or 3.8 mg intramuscularly (im), $n = 20$, or placebo, $n = 20$, using a needle-free injection device. DNA plasmids encoding HIV-1 genes *gp160* subtype A, B, C; *rev* B; p17/p24 *gag* A, B and *Rtmut* B were given at weeks 0, 4 and 12. Recombinant MVA (10^8 pfu) expressing HIV-1 Env, Gag, Pol of CRF01_AE or placebo was administered im at month 9 and 21.
Results: The vaccines were well tolerated. Two weeks after the third HIV-DNA injection, 22/38 (58%) vaccinees had IFN-γ ELISpot responses to Gag. Two weeks after the first HIV-MVA boost all 35 (100%) vaccinees responded to Gag and 31 (89%) to Env. Two to four weeks after the second HIV-MVA boost, 28/29 (97%) vaccinees had IFN-γ ELISpot responses, 27 (93%) to Gag and 23 (79%) to Env. The id-primed recipients had significantly higher responses to Env than im recipients. Intracellular cytokine staining for Gag-specific IFN-γ/IL-2 production showed both $CD8^+$ and $CD4^+$ T cell responses. All vaccinees had HIV-specific lymphoproliferative responses. All vaccinees reacted in diagnostic HIV serological tests and 26/29 (90%) had antibodies against gp160 after the second HIV-MVA boost. Furthermore, while all of 29 vaccinee sera were negative for neutralizing antibodies against clade B, C and CRF01_AE pseudoviruses in the TZM-bl neutralization assay, in a PBMC assay, the response rate ranged from 31% to 83% positives, depending upon the clade B or CRF01_AE virus tested.

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* Corresponding author. Tel.: +255 754 387328; fax: +255 222 151350.
E-mail addresses: drbakari@yahoo.com, mbakari@muhas.ac.tz (M. Bakari).

[1] Authors contributed equally to this report.
[2] For the HIVIS study group.

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This vaccine approach is safe and highly immunogenic. Low dose, id HIV-DNA priming elicited higher and broader cell-mediated immune responses to Env after HIV-MVA boost compared to a higher HIV-DNA priming dose given im. Three HIV-DNA priming immunizations followed by two HIV-MVA boosts efficiently induced Env-antibody responses.

1. Introduction

Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) is a global burden with an estimated 2.6 million new HIV infections and 2.0 million HIV related deaths in 2009 [1]. Sixty-seven percent of all people living with HIV are found in sub-Saharan Africa, and 72% of total global AIDS deaths occurred in the region in 2009 [1]. Approximately 60% of HIV infections in sub-Saharan Africa occur among women. HIV infection and AIDS is currently the major cause of adult mortality and ill health in sub-Saharan Africa necessitating the need for new preventive strategies to reduce the burden of HIV transmission [1]. An effective prophylactic vaccine would offer one such preventive strategy to complement other efforts, but has proven difficult to develop [2].

Vaccine development initially focused on induction of antibody responses using recombinant monomeric envelope proteins. These proved immunogenic but gave no protection in two phase III studies in the USA and Thailand [3]. The difficulties in eliciting broadly neutralizing antibodies to HIV [4] have prompted alternative vaccine approaches that focus on the induction of cell-mediated immune responses. Studies in non-human primate models using SHIV and SIV DNA vaccines and live vector based vaccines such as adenovirus (Ad5) or recombinant modified vaccinia virus Ankara (MVA) in prime boost regimens have shown that this approach is effective in reducing challenge virus replication and preventing the development of SIV-induced disease [5–8]. Recombinant DNA and Ad5 or poxvirus vector-based HIV-1 vaccine candidates have been evaluated alone or in prime boost regimens in several phase I and phase II trials and shown varying levels of immunogenicity [9–26].

Vaccination with a clade B Ad 5-based HIV-1 vaccine in a phase IIB clinical trial (the STEP trial) was discontinued in 2007 because the vaccine was not effective and there was a trend towards increased rates of HIV acquisition among vaccinated men who had pre-existing Ad5-antibody titres and/or were uncircumcised [27,28]. A phase IIB study is ongoing in circumcised men without prior Ad5 neutralizing antibody titres to assess the safety and effect on post-HIV acquisition viremia of a multiclade multigene DNA vaccine boosted with the same genes in a recombinant Ad5 vector vaccine (HVTN 505) [29].

The hope for an HIV vaccine has been strengthened by the recent finding in the RV 144 trial that a canary pox prime with *env*, *gag* and *pol* genes, boosted by an Env protein vaccine that in itself did not provide protection in men who have sex with men or injection drug users [30], protected 31.2% (95% CI 1.1–52.1%, $p = 0.04$) of largely heterosexual Thais with a low risk of HIV exposure. [31]. Another prime boost concept with the use of HIV DNA for priming and recombinant vaccinia virus for boosting has been pursued by a number of groups. Initial studies showed low immunogenicity [14]. However, a trial of homologous multigene clade C DNA prime/NYVAC boost has shown promising results [15,16], as did a B clade multigene DNA/MVA vaccine [24].

However, DNA vaccines have been found to be poor immunogens and require high concentrations of DNA when given intramuscularly. More efficient delivery methods and better immunogens are therefore needed. A randomized, open label, phase I HIV-1 vaccine study (HIVIS01/02) was performed in Stockholm, Sweden, to assess different modes of administering an HIV DNA vaccine candidate (plasmid DNA with inserted HIV genes *env*, *rev*, *gag* and *RT*) boosted with heterologous HIV-1 recombinant modified vaccinia virus Ankara (MVA) with analogous genes. The results were promising: 34 out of 38 vaccinees had HIV-specific IFN-γ enzyme-linked immunospot (ELISpot) responses and 35 of 38 vaccinees had a positive lymphoproliferative assay (LPA) response. Overall, 37 of 38 vaccinees (97%) were responders. Importantly, a low dose of HIV-DNA administered intradermally (id), was as effective as a high dose intramuscularly (im), in priming for the MVA boosting vaccine [17].

Preparations for HIV-1 vaccine development in Tanzania have been underway since 1994. They have included studies of a possible cohort for vaccine trials consisting of police officers (POs) in Dar es Salaam [32], determination of prevalent HIV-1 subtypes in Dar es Salaam and Mbeya, which has motivated the inclusion of subtypes A, C and B (as a substitute for subtype D) in the vaccine [33,34]; training of laboratory and clinical personnel; and development and establishment of virological and immunological methods for vaccine immunogenicity assays in the collaborating laboratories in Sweden and Tanzania.

Based on the HIVIS01/02 phase I study, we have conducted a phase I/II clinical trial (HIVIS03) among healthy HIV negative volunteers in Dar es Salaam, Tanzania. The aim has been to continue the evaluation of safety and immunogenicity of low dose id priming, compared to higher dose im priming, with the HIVIS multigene, multiclade HIV-1 plasmid DNA vaccine followed by a MVA boost with heterologous HIV-1 inserts.

2. Methods

2.1. Study vaccines

Volunteers were immunized with HIVIS DNA plasmids encoding Env of HIV-1 subtypes A, B and C; Gag of subtypes A and B, and RT and rev of subtype B, followed by a boost with recombinant MVA-CMDR encoding Env of HIV-1 subtype E and Gag–Pol of subtype A at months 9 and 21 [17,35–37]. The seven DNA plasmids were delivered as two entities; one containing the Gag- and RT-encoding plasmids, the other containing the gp160 Env A–C and rev-encoding plasmids, in order to avoid immunodominance of either combination [38]. The DNA vaccine was produced by Vecura at the Karolinska Hospital, Sweden. Recombinant Modified Vaccinia virus Ankara, MVA-CMDR (HIV MVA) has been described before [39]. The construct expresses HIV-1 subtype E Env and subtype A Gag/Pol from Thai isolates CM235 and CM240 both under control of the early/late mH5 promoter. The cytoplasmic tail of Env was truncated and the *RNaseH* and *integrase* genes were entirely deleted. In addition, the active site of RT contains a mutation that abolishes enzymatic activity. The vaccine was produced by the WRAIR Pilot Bio production facility, Forest Glen, MD, USA.

2.2. Study design and population

This randomized, double-blind, placebo-controlled phase I/II trial comparing id and im administration of the DNA prime with Biojector was conducted in Dar es Salaam, Tanzania. The study had a power of 90% to show that the event rate for the id group is the same as the event rate for im group with a sample size of 20 in both groups. This assumed that a difference of 20.0 points or less is unimportant and that alpha (1 tailed) is set at 0.05.

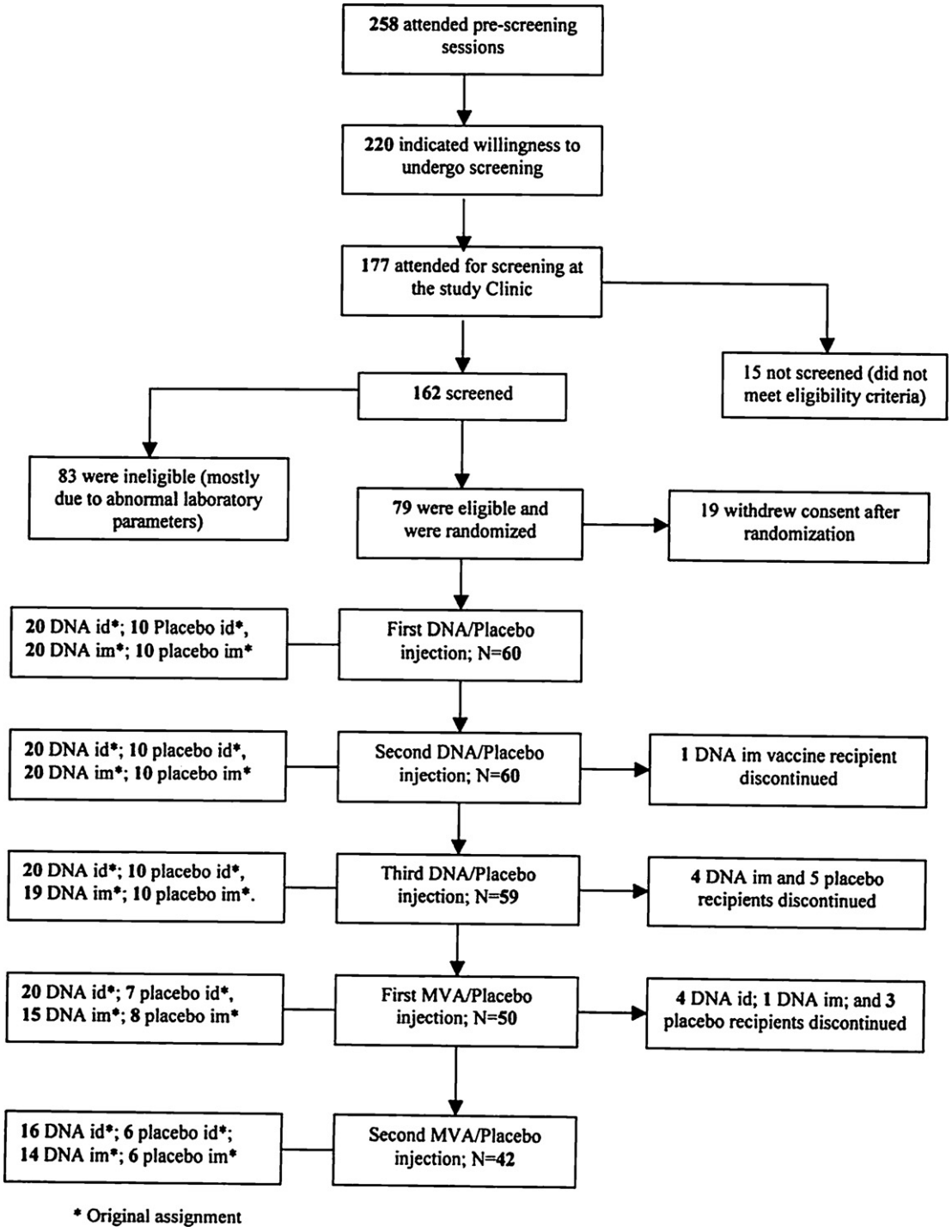

Fig. 1. Profile of trial participants showing 258 who attended pre-screening sessions, 162 screened to obtain 60 enrolled, 59 who received three HIV-DNA/placebo vaccinations, 50 who received the first HIV-MVA/placebo vaccinations and 42 who received the second HIV-MVA/placebo vaccinations.

Until the end of study, the volunteer, the clinic and the laboratory remained blinded as to whether the volunteer received vaccine or placebo. Consenting healthy volunteers at low risk of HIV-1 infection from a Police Officers' cohort were recruited for the trial. The overall HIV-1 prevalence and incidence in 1994–1996 were found to be 13.8% and 1.96%, respectively [32].

The extensive interaction with the Police Force enabled us to enroll into the trial individuals with as low HIV infection risk as possible within this population and facilitate follow-up. Hierarchical coercion for participation in the clinical trial was not an issue, as the Police Authorities and POs fully accepted that volunteers participated as independent individuals.

2.3. Recruitment of study volunteers

Potential volunteers (Fig. 1) were invited to pre-screening sessions at which the study plan was presented. Those indicating a willingness to participate were invited to a first screening at the study clinic at which a written informed consent was obtained. The volunteers who were between 18 and 40 years of age, healthy,

Table 1
Randomization groups by dose and route of HIV-DNA injections and HIV-MVA boosting vaccination.

Treatment group	HIV-DNA priming vaccination		HIV-MVA boosting vaccination
	Left arm, *env*	Right arm, *gag* and *Rtmut*	
II ($n=20$)	3 id injections of 100 μl; total 0.6 mg DNA	2 id injections of 100 μl; total 0.4 mg DNA	im injection of 10^8 pfu
IIIB ($n=10$)	3 id injection of saline	2 id injection of saline	im injection of saline
I ($n=20$)	1 im injection of 1.0 ml; total 2.0 mg DNA	1 im injection of 0.9 ml; total 1.8 mg DNA	im injection of 10^8 pfu
IIIA ($n=10$)	1 im injection of saline	1 im injection of saline	im injection of saline

All DNA injections were given by a needle-free injection system (Biojector 2000, Bioject Medical Technologies).

at low risk of STI/HIV, and not planning to conceive a child for the specified period as per protocol were invited to a second screening visit 2–4 weeks later. Contraceptive methods included education and provision of condoms for consistent use as well as other methods preferred by the volunteer. Contraception was to be observed during the entire study duration and 4 months following the last vaccination. At this second screening, a second informed consent for enrolment into the trial was obtained, and randomization was done. The random allocation was performed by the Study Pharmacist opening consecutively numbered sealed envelopes (containing a random assignment to the groups). The first immunization took place within 4 weeks after randomization. Nineteen (24.1%) volunteers exited after randomization but before the first injection; nine of them indicated that their "significant others" (i.e. spouse or parents) did not favour their participation, seven did not give a reason, and three were not accessible. Their assignments were re-randomized to others and the screening process continued until the 60 intended volunteers had been enrolled.

2.4. Randomization and vaccination of study volunteers

The volunteers were randomized to four groups by the study pharmacist by breaking consecutively numbered envelopes containing random assignments; 30 were randomized to id DNA/placebo administration of whom 20 were allocated to vaccine and 10 to placebo and 30 were randomized to im of whom 20 were allocated to vaccine and 10 to placebo. The clinic was blinded to the vaccine/placebo allocation but informed of the injection modality (Table 1). All DNA injections were given by a needle-free Biojector device (Bioject Medical Technologies, Inc., Tualatin, OR, USA). At each id immunization at weeks 0, 4 and 12, three injections of 0.1 ml were given in the skin over the left deltoid and two injections of 0.1 ml over the right deltoid for a total of 1.0 mg per immunization. The total id dose was thus 3 mg. The im immunization was given in the deltoid muscles for a total of 3.8 mg per immunization. The total im dose was thus 11.4 mg. The immunogens were divided so that the *env/rev* plasmids were given in the left arm and the *gag/pol* plasmids in the right arm.

A boost of 10^8 pfu of recombinant HIV-MVA or placebo was administered im at month 9 using a needle and syringe to fifty volunteers who had completed the three HIV-DNA/placebo injections and a second HIV-MVA/placebo boost was given at month 21 to the 42 eligible volunteers. The interval between the first and second HIV-MVA/placebo was determined by the availability of vaccine. The volunteers were given 1 ml containing 10^8 pfu im of HIV-MVA or placebo by needle injection in the left deltoid muscle (Table 1).

2.5. Clinical safety assessments

Clinical events were actively investigated on the basis of a seven-day diary card provided at each immunization visit and by interviews at the clinic, two weeks after each HIV-DNA or HIV-MVA immunization and recorded by symptom. Safety assessments were also done at visits 4, 8 and 24 weeks after the HIV-MVA immunizations.

2.6. Laboratory baseline and safety assessments

Samples for safety analysis were obtained 4–8 weeks before enrolment, 2 weeks after each immunization, and 4 and 24 weeks after HIV-MVA immunizations, as well as when clinically indicated. In addition, active hepatitis B infection and syphilis were excluded at the first screening visit. Twelve-lead electrocardiograms (ECG) were performed before and two weeks after the HIV-MVA/placebo immunizations in order to comply with current US-FDA recommendations to monitor for potential peri-myocarditis due to MVA [40,41].

The baseline and safety laboratory tests included a complete blood count (CBC), alanine aminotransferase (ALT), total and direct bilirubin, creatinine, fasting blood glucose and a complete urine analysis including pregnancy test for female participants. CBC was performed using AcT5 Diff analyzer (Beckman Coulter, USA) while safety tests were performed using Cobas Integra 400 Plus analyzer (Roche Diagnostics, USA). Counting of T-lymphocyte subsets was performed using FACSCalibur (Becton Dickinson, NJ, USA). All the laboratory tests were done in the Department of Microbiology and Immunology at Muhimbili University of Health and Allied Sciences (MUHAS). The laboratory participates in several external proficiency testing programmes including College of American Pathologists (CAP), United Kingdom National External Quality Assurance Scheme (UKNEQAS) and USA Virology Quality Assurance (VQA).

2.7. Cellular immunogenicity assessment

2.7.1. Blood collection and processing of peripheral blood mononuclear cells (PBMC)

Whole blood samples for analysis of cell-mediated immune responses were collected in vacutainer tubes containing sodium heparin as anticoagulant and were processed within 6 h as previously described [42]. Peripheral blood mononuclear cells (PBMC) were purified using LeucoSep tubes according to the manufacturers' instructions (Greiner Bio-One). PBMC yield and viability were determined using a NucleoCounter (ChemoMetec A/S, Allerød, Denmark). Fresh PBMCs were used for ELISpot, intracellular cytokine staining (ICS) and LPA. The remaining cells were cryopreserved in a cryoprotectant medium containing 90% foetal calf serum (FCS) and 10% DMSO in a Mr Frosty container (Nalgene). Cells were stored in liquid nitrogen containers.

2.7.2. IFN-γ ELISpot assay

IFN-γ ELISpot assay was performed using the h-IFN-gamma ELISpotPLUS kit in a two-step detection system according to the manufacturer's instructions (Mabtech, Nacka, Sweden) as previously described [42]. Briefly, pre-coated IFN-γ ELISpot plates were washed and phyto-haemagglutinin (PHA, positive control), a peptide pool (CEF) composed of a panel of 23 peptides from cytomegalovirus (CMV), Epstein–Barr virus and influenza virus [43], a peptide pool of 138 peptides (15 mers with an overlap of 11 amino acids) spanning the pp65 protein of human CMV (PepMix, JPT, Berlin, Germany) and HIV-1 specific peptide pools

Table 2
HIV-specific peptide pools used in ELISpot assays.

Peptide pool ID	Protein	Peptide number	Clade
Gag I SMI[b]	p17	1–26	B
Gag II SMI[b]	p24	27–71	A
Env I SMI[b]	gp120, including V1 and V2	1–50	A/B
Env II SMI[b]	gp120, including V3–V5	51–100	A
Env III SMI[b]	gp41	101–169	B
Gag WR[a,c]	p6, p7, p17, p24	1–160	A
Env WR[a,c]	Env	1–177	E
Pol WR[a,c]	Pol	1–146	A

All peptides were 15-mers with 10 amino acid (aa) overlap except in.
[a] WR peptide pools which had peptides with 11 aa overlap.
[b] HIV-1 vaccine clade A and B-specific peptides corresponding to the DNA prime.
[c] HIV-1 vaccine-specific peptides corresponding to the MVA boost.

(Table 2) were diluted in complete RPMI medium and added in triplicate (50 μl/well). A final concentration of 5 μg/ml was used for PHA and CEF while 2.5 μg/ml was used for all HIV-DNA vaccine-specific peptide pools. The pp65 CMV peptide pool and all HIV-MVA vaccine-specific peptide pools were used at 1 μg/ml. RPMI medium was added in triplicate and used as background control. Fifty microliters cell suspension of freshly isolated PBMC was added to each well giving 200,000 cells/well. The plates were incubated for 20 h at 37 °C, with 7.5% CO_2. Frequencies of antigen-specific spot-forming cells (SFC) were measured in an automated Immunospot analyzer (CTL-Europe, Bonn, Germany). Results were expressed as SFC per million PBMC and were calculated for each pool of peptides as follows: 5 × the mean SFC from three stimulated wells, without subtracting background. ELISpot responses were considered positive if the number of SFC was >55 spots/10^6 peripheral blood mononuclear cells and 4 times the background and the baseline value. Data where background responses in three medium wells exceeded a median of 60 per million PBMCs were excluded from analyses.

2.7.3. 4-colour ICS assay

For the determination of $CD4^+$ and $CD8^+$ T cell responses, a 4-colour ICS assay was performed on fresh PBMC, which had been rested overnight following the purification procedure. PBMC (0.5×10^6) were incubated in 96-well round-bottom plates with co-stimulatory anti-CD28 (1 μg/ml) and anti-CD49d (1 μg/ml) monoclonal antibodies (Becton Dickinson, BD Pharmingen, San Diego, CA), in either medium only (negative control) or in medium containing a mixture of staphylococcal enterotoxin A and B (SEAB, 1 μg/ml) (Sigma, St. Louis, MO), CEF peptide pool (1 μg/ml), CMV peptide pool (PepMix, 0.5 μg/ml), HIV-1 Gag-specific peptide pools (Table 2) and brefeldin A (0.5 μg/ml) (Sigma, St. Louis, MO). Samples were incubated for 6 h at 37 °C in a 7.5% CO_2 incubator and were stored at 4 °C overnight. The next day, 20 μl (20 mM) of EDTA was added to each well and incubated for 15 min. Thereafter, the samples were transferred to a V-bottom plate and centrifuged for 5 min. Cells were washed twice in wash buffer (PBS + 0.1% FCS), permeabilized in BD Cytofix/Cytoperm buffer for 20 min at 4 °C (dark) and washed once in BD Perm/Wash buffer. Cells were then stained for 30 min at 4 °C with an antibody cocktail containing anti-CD3–APC, anti-CD4–FITC, anti-CD8–PerCpCy5.5, anti-IFN-γ-PE and anti-IL-2-PE (Becton Dickinson, San Jose, CA). At the end of the incubation cells were washed twice in Perm/Wash buffer, fixed in BD CellFix solution and stored at 4 °C in the dark until acquisition. Acquisition of samples was performed using a FACSCalibur flow cytometer and samples were analyzed using FlowJo software, version 8.7.1 (Tree Star, Ashland, OR). A minimum of 70,000 $CD3^+$ lymphocytes per well was required for a sample to be included in the analysis. Background levels were established using Tanzanian blood donor samples ($n = 19$). ICS responses were considered positive if they were at least 3-fold higher than the mean of background (medium samples) and above 0.05% for $CD4^+$ T lymphocytes and above 0.1% for $CD8^+$ T lymphocytes.

2.7.4. Tritiated [^{3}H]-thymidine LPA

The LPA was performed using freshly isolated PBMCs, which were cultured in triplicate with or without HIV antigen, or PHA in complete medium in 96-well flat-bottomed plates (Nunclon, Aahus, Denmark) at 37 °C, 7.5% CO_2 as described previously [44]. Cell cultures were pulsed for 6 h on day 2 (PHA) and day 6 (antigens) with 1 μCi [^{3}H]-thymidine per well (GE-Healthcare Bio-Sciences AB, Uppsala, Sweden). The antigens used at a final concentration of 2.5 μg/ml were aldrithiol-2 (AT-2) treated HIV-1 CM235 (subtype CRF01_AE) and Jurkat Tat CCR5 microvesicles (control), kindly provided by Dr. J. Lifson, SAIC Frederick, Inc., Frederick, USA. Thymidine incorporation was measured in a 1450 MicroBeta liquid scintillation counter (Wallac, Turku, Finland). T cell proliferation was reported as a stimulation index (SI), determined by dividing the mean counts per minute of the antigen-stimulated wells by the mean of the unstimulated control wells. A SI above six was considered positive, based on the mean reactivity at baseline in 57 volunteers.

2.8. Humoral immunogenicity assessment

2.8.1. Assessment of binding antibodies

Antibodies to native gp160 (IIIB, Advanced Biotechnologies Inc.) were tested using an in-house enzyme-linked immunosorbent assays (ELISA). Diagnostic HIV serological testing was performed using the Murex HIV antigen/antibody (Abbott Murex, UK) and the Enzygnost® anti-HIV-1/2 Plus (Dade Behring, Marburg, Germany) ELISAs. Samples that gave repeated discordant results between Murex and the Enzygnost assays were resolved using confirmatory Inno-Lia immunoblot assay (Inno-genetics, Belgium) [45].

2.8.2. Neutralization assay

TZM-bl pseudovirus neutralization assay: Neutralizing antibody (NAb) was measured using pseudoviruses and a luciferase based assay in TZM-bl cells as previously described [46]. The assay measures the reduction in luciferase reporter gene expression in TZM-bl cells with a single round of pseudovirus infection. Briefly, 200 TCID50 virus (25 μl) was incubated with human serum (1:20 dilution) for 1 h at 37 °C in 96-well black flat bottom plates, followed by addition of 1×10^4 TZM-bl cells in a 50 μl volume of DMEM medium containing a final concentration of 40 μg/ml DEAE-dextran. Each plate included replicate wells of cells alone (background control) and cells with virus (virus control). After 48 h incubation, 100 μl of Britelite substrate was added into all wells and luminescence was measured using a Victor Light luminometer (Perkin Elmer). The % inhibition was then calculated as compared to relative luminescence units (RLU) in the virus control wells, after subtraction of background (cells alone) RLU. A result ≥ 50% is considered to be a positive response.

PBMC neutralization assay: A PBMC assay employing an infectious molecular clone (IMC) that carry a LucR gene as a reporter was used [47]. IMC reporter virus (25 μl) was incubated at 37 °C with 25 μl of diluted test serum from pre-and post-vaccination (1:60 dilution) in duplicates or triplicates in 96-well round bottom plates for 1 h. PHA stimulated PBMC (1.5×10^5 cells/well in 50 μl) were added and plates were incubated overnight. cRPMI/IL-2 medium (100 μl) was added to each well on the next day and plates were incubated further for three days. Lysis buffer (50 μl) was added, plates were subjected to two freeze/thaw cycles, 20 μl of the cell lysate was transferred to a corresponding 96-well black plate, and 100 μl of substrate were added into each well via the Envision Luminometer (Perkin Elmer) injection system, followed

by immediate measurement of luminescence in RLU. The percent neutralization by post-vaccination serum was calculated based on the level of virus growth in the presence of the same dilution of pre-vaccination serum and a result ≥ 50% was considered a positive response.

2.9. Data analysis

Clinical and safety laboratory data were double entered in Access database, while the laboratory immunological and HIV serology data were entered in Excel program. Clinical, safety laboratory and immunological data were merged and analyzed in SPSS 17.0 under a separate code to avoid premature un-blinding after both databases were frozen. Cross-tabulations were evaluated with Fisher's Exact test or Chi-square as appropriate. Means and medians were compared with Students *t*-test or Mann–Whitney as appropriate. Pearson's correlation coefficient was used for examining the strength of association between IFN-γ ELISpot and LPA results. $p < 0.05$ (double sided) was considered significant. The clinic and laboratory personnel as well as the volunteers were blinded as to volunteer immunization assignments until after completion of the study.

2.10. Ethics statement

The study protocol was approved by Tanzania's National Health Research Ethics Committee and the Senate Research and Publications Committee of MUHAS; use of the vaccine candidate products on humans in Tanzania was approved by the Tanzania Food and Drugs Authority (TFDA). The study was conducted in accordance with the International Conference on Harmonization, Good Clinical Practice guidelines (ICH-GCP). All volunteers provided signed written informed consent.

3. Results

3.1. Enrolment and demographics

Two hundred and fifty eight volunteers attended the pre-screening sessions and 220 (85%) were interested to undergo screening (Fig. 1). Of these, 162 (74%) were screened over a 12-month period, beginning in January 2007. Eighty-nine (50%) volunteers were ineligible for recruitment, mainly on account of abnormal laboratory test results based on reference ranges for Dar es Salaam's healthy adult population. Sixty volunteers were enrolled, of whom 15 (25%) were females (Fig. 1); the Police Force in Dar es Salaam is largely male. The overall median age for the enrolled volunteers was 28 years; all but one male (age 47, who was enrolled before an age restricting amendment based on findings from HIVIS-01 trial) were between 20 and 40 years. The median age by gender was 28 years for males, and 25 years for females ($p = 0.451$). Scars compatible with previous vaccinia vaccination were found in 32 (54%) of the 60 individuals. Vaccine and placebo recipients had similar baseline demographic characteristics, and there was excellent adherence to the scheduled visits to the trial clinic by the volunteers.

One enrolled volunteer was excluded from further immunizations due to a rising serum creatinine level and an elevated blood pressure after receiving the second HIV-DNA im injection. The condition was in retrospect found to have been present at enrolment, when the creatinine level was just below the upper normal limit and hence was considered to be unrelated to the vaccine. Four other volunteers who had received DNA im and 5 who received placebo were not eligible for the HIV-MVA/placebo boost; the reasons were social (4), pregnancy (1), and medical (4). The medical reasons were; a pre-existing partial seizures disorder, a pre-existing ovarian tumour, and urticaria in three im vaccine recipients; and a sickle-cell trait associated anaemia in one id placebo recipient. None of the reasons for ineligibility to receive the HIV-MVA/placebo boost were related to the HIV-DNA vaccine/placebo or the mode of injection. Forty two volunteers received a second HIV-MVA/placebo injection at month 21. Four recipients of id vaccine (one each due to self withdrawal, wanted pregnancy, pregnancy, HIV infection), one im vaccine recipient (pregnancy), and three placebo recipients (one each; transferred from Dar es Salaam, anaemia, suspected arteritis) were not eligible.

3.2. Safety and tolerability

The HIV-DNA and HIV-MVA vaccines were well tolerated. A total of 119 adverse events (AEs) were reported within 2 weeks of the three DNA/placebo administrations. Forty-eight (40%) of these occurred in id vaccine, 43 (36%), in im vaccine and 28 (24%) in placebo recipients. Most were mild, but 8 (4 id vaccine, 3 im vaccine and 1 placebo) were considered moderate. Only one event (headache), in a placebo recipient, was deemed probably related to vaccination. Furthermore, one severe adverse event (SAE) was recorded in a placebo recipient. The most common events were headache (25 (21%) of which 10 were in id vaccine, 7 in im vaccine and 8 in placebo recipients) and local pain (14 (12%) [4, 9 and 1, respectively]). However, there was no increase in severity of the events over time.

A total of 47 events were reported within 2 weeks of the two HIV-MVA/placebo injections. Thirty six (77%) of these occurred in HIV-MVA and 11 (23%) in placebo recipients. All but one were mild; one HIV-MVA recipient had a moderate reaction of local pain, which was possibly related to vaccination. The most common AE was local pain (12 (30%) of which 11 were in MVA, and 1 in placebo recipients). There was no increase in severity over time.

Eleven SAEs occurred during the study period up to visit 24, i.e. about 6 months after the last MVA injection. Out of these SAEs, one (acute abdominal pain due to severe constipation) in a placebo recipient, and one (hematemesis) in a MVA recipient occurred within two weeks after the third HIV-1 DNA/Placebo and second MVA immunizations, respectively. They were considered to be unrelated to vaccination. The rest of SAEs occurred more than two weeks after the immunizations. One occurred after the first id DNA vaccination (injury in a motor vehicle accident); 3 after the third im DNA vaccination (fainting, fissure in ano, mild head injury); 4 after the first MVA vaccination (musculoskeletal chest pain, epistaxis, acute gastroenteritis, paralytic ileus); and 1 in a placebo recipient after the second MVA/placebo vaccination (hemoptysis). Two were deemed probably not related, while the remaining were unrelated.

During the follow up for vaccination safety, none of the volunteers developed significant changes from baseline values of laboratory parameters (serum ALT, serum total and direct bilirubin, serum creatinine, total WBC count, lymphocytes count, haemoglobin, platelets and granulocyte counts (neutrophils, eosinophils, basophils counts). Furthermore, no MVA related ECG abnormalities were noted.

3.3. Cellular immunogenicity

3.3.1. IFN-γ ELISpot responses

Table 3 summarizes the IFN-γ ELISpot response rates to the various HIV peptide pools in vaccinees after the third HIV-DNA immunization and after the first and second HIV-MVA boosts, and Fig. 2 shows the magnitude of the HIV-specific IFN-γ ELISpot responses in the responders. Two weeks after the third HIV-DNA injection, 22 (58%) of the 38 vaccinees had IFN-γ ELISpot responses to Gag. Twelve (63%) of 19 id vaccinees and 9 (47%) of 19 im

Table 3
Summary of interferon γ ELIspot response rate to various HIV peptide pools in vaccinees.

Peptide pool	Two weeks after three HIV-DNA immunizations Responders, no. (%)		Two weeks after the first HIV-MVA boost Responders, no. (%)		Two to four[a] weeks after the second HIV-MVA boost Responders, no. (%)	
	id[b] (n = 19)	im[c] (n = 19)	id (n = 20)	im (n = 15)	id (n = 15[d])	im (n = 14)
Gag I	8 (42)	7 (37)	15 (75)	7 (47)	5 (33)	6 (50)
Gag II	10 (53)	6 (32)	20 (100)	13 (87)	13 (87)	11 (79)
Gag WR	7 (37)	8 (42)	20 (100)	15 (100)	11 (73)	12 (86)
Env I	0 (0)	0 (0)	13 (87)	3 (20)	5 (33)	1 (7)
Env II	0 (0)	0 (0)	4 (20)	1 (7)	2 (13)	1 (7)
Env III	0 (0)	0 (0)	10 (50)	4 (27)	5 (33)	3 (21)
Env WR	0 (0)	0 (0)	17 (85)	12 (80)	11 (73)	10 (71)
Pol WR	1(5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Any Gag	13 (68)	9 (47)	20 (100)	15 (100)	14 (93)	13 (93)
Any Env	0 (0)	0 (0)	19 (95)	12 (80)	13 (87)	10 (71)
Gag or Env	13 (68)	9 (47)	20 (100)	15 (100)	15 (100)	13 (93)

[a] Four week data were used in 3 cases for which two-week data were unavailable.
[b] HIV-DNA priming immunization given id.
[c] HIV-DNA priming immunization given im.
[d] One additional volunteer who received the HIV-DNA priming immunization id was not evaluable.

vaccinees reacted to one or both of the HIV-DNA vaccine-specific Gag peptide pools (Gag I and Gag II). One additional id volunteer responded to the GagWR pool. However, the magnitude of the immune responses was modest. The median responses in responders to Gag I (p17) and Gag II (p24) following id vaccine administration were 88 and 108 SFC/million PBMCs, respectively. The corresponding responses following im vaccine administration were 180 and 160 SFC/million PBMCs, respectively. None of the vaccinees responded to any of the Env peptide pools (Fig. 2A and B).

Thirty-five HIV-DNA vaccine recipients were given a first HIV-MVA boost, and 30 received a second boost. Two weeks after the first HIV-MVA boost, all 35 (100%) vaccine recipients had IFN-γ ELISpot responses: 35 to Gag and 31 of 35 (89%) to any of the Env peptide pools. In four of 30 vaccinees, the INF-γ ELIspot assay was non-valid two weeks after the second HIV-MVA boost; and in 3 of them the IFN-γ ELISpot results obtained four weeks after the second HIV-MVA boost were used in the calculation of the response rate and the magnitude of responses (one vaccinee had non-valid results also after the second HIV-MVA boost). Two to four weeks after the second HIV-MVA boost, 28 (97%) of 29 evaluable vaccinees had positive responses, 27 (93%) to Gag and 23 (79%) to Env (Table 3).

After the first HIV-MVA boost, all 35 (100%) vaccinees responded to the Gag WR peptide pool, whereas after the second HIV-MVA boost this was the case for 23 of the 29 (79%) evaluable vaccines. Three of the non-responders to Gag WR had positive responses to Gag II and one to Gag I. IFN-γ ELISpot responses to Gag WR were significantly higher after the first than after the second HIV-MVA boost (median 625 vs 335 SFC/million PBMC, $p = 0.005$, in all the 29 evaluable vaccinees; and median 1005 vs 525 SFC/million PBMC, $p = 0.048$, in the 23 vaccinees who had positive responses to Gag WR after both the first and the second boost). In contrast, the magnitude of the responses to the Env WR peptide pool was not significantly different after the first and second HIV-MVA boost (median 267 and 232 SFC/million PBMC, respectively, $p = 0.786$, in all the 24 vaccinees who had positive Env WR responses after the first HIV-MVA boost and evaluable IFN-γ ELISpot tests after the second HIV-MVA boost; median 278 and 275 SFC/million PBMC, respectively, $p = 0.780$ in the 20 vaccinees who were Env WR responders at both time points). After the first and second HIV-MVA boosts, responses to Gag WR did not differ significantly between volunteers previously DNA primed id compared to im. Median responses to Gag WR were 590 ($n = 20$) and 715 ($n = 15$) SFC/million PBMC, respectively, after the first boost ($p = 0.777$) and 550 ($n = 11$) and 337 ($n = 12$) SFC/million PBMC, respectively, after the second boost ($p = 0.479$). However, the responses to the Env WR peptide pool matching the boost were significantly higher in id compared to im DNA-primed vaccinees. Median responses to Env WR were 415 ($n = 17$) and 187 ($n = 12$) SFC/million PBMC, respectively, after the first boost ($p = 0.003$) and 445 ($n = 11$) and 195 ($n = 10$), SFC/million PBMC, respectively, after the second boost ($p = 0.035$) (Fig. 2). After the first HIV-MVA boost, more id DNA primed volunteers (13/20) reacted against the N-terminal of gp120 (peptide pool Env I) compared to those primed im (3/15, $p = 0.016$). Furthermore, more volunteers in the id group had reactions to multiple peptide pools compared to the im group. Of the 20 id primed volunteers, 14 (70%) reacted to 3 or more of the complementary peptide pools matched to the HIV-DNA vaccine, indicating that at least that many different epitopes were recognized; the corresponding number for the im primed vaccinees was 4/15, 27% ($p = 0.006$) (Table 4). None of 15 placebo recipients had a positive IFN-γ ELISpot response two weeks after the fourth placebo injection, which corresponded in time to the first HIV-MVA immunization in the vaccinees. However, two weeks after the fifth placebo injection, one volunteer had a response to Env II and another placebo recipient had a response to Env WR.

Age influenced the immune responses of the volunteers. There was a lower specific immune response induced to GagWR pool by age ($r^2 = 0.168$, $p = 0.014$). Furthermore, there was no significant difference in immune response by gender (data not shown). The presence of vaccinia scars did not influence the response rate.

Table 4
The number of reactive volunteers in IFN-γ ELISpot to the complementary peptide pools specific for the HIV-1 DNA vaccine (Gag I, Gag II, Env I, Env II and Env III) 2 weeks after the first HIV-MVA boost by priming group.

HIV-DNA priming group	Responders to number of peptide pools matched to the DNA vaccine						Total
	0	1	2	3	4	5	
Id	0	3	3	6	5	3	20
Im	1[a]	4	6	4	0	0	15
Total	1	7	9	10	5	3	35

[a] One volunteer was only reactive to the Gag WR peptide pool.

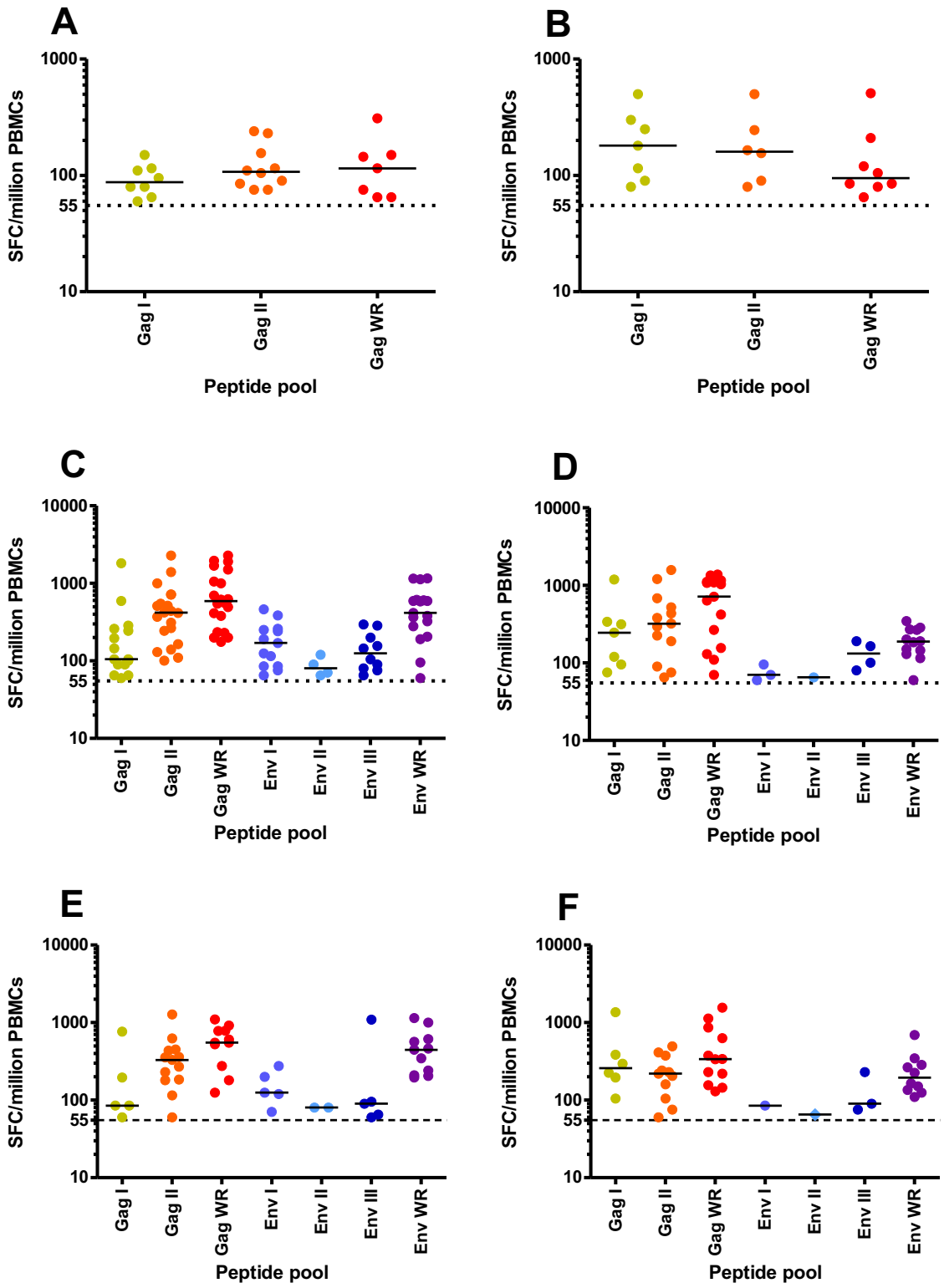

Fig. 2. HIV-specific interferon-γ ELISpot responses in responders (cut-off SFC was >55 spots/10^6 peripheral blood mononuclear cells and 4 times the background and the baseline value): (a) two weeks after three HIV-DNA injections by id group; (b) two weeks after three HIV-DNA injections by im group; (c) two weeks after the first HIV-MVA boost in the DNA id-primed group and (d) two weeks after the first HIV-MVA boost in the DNA im-primed group; (e) two to four weeks after the second HIV-MVA boost in the DNA id-primed group and (f) two to four weeks after the second HIV-MVA boost in the DNA im-primed group.

3.3.2. 4-colour ICS responses

HIV-specific $CD4^+$ and $CD8^+$ T cell responses to Gag were determined by an IFN-γ/IL-2 ICS assay four weeks after the second HIV-MVA boost. Of the 29 vaccinees, 25 (86%) had $CD4^+$ and/or $CD8^+$ T cell responses to any of the HIV Gag peptide pools, 16 (55%) in the $CD4^+$ T cell compartment and 17 (59%) in the $CD8^+$ T cell compartment. The Gag-specific response rate and the magnitude of the responses did not differ significantly between the id (13/15) and im vaccinees (12/14). The magnitude of the $CD4^+$ and $CD8^+$ T cell responses is shown in Fig. 5. The median (range) IFN-γ/IL-

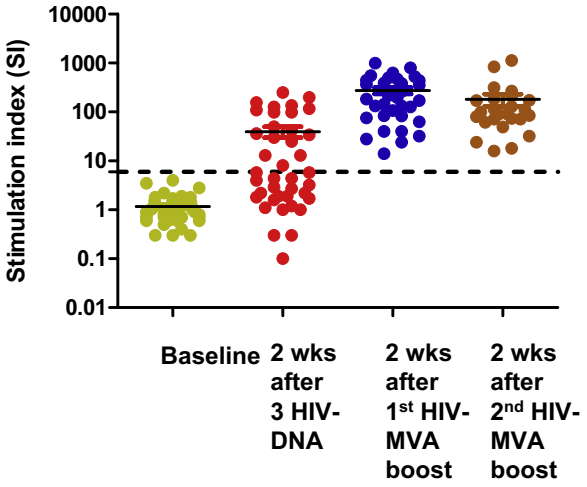

Fig. 3. Lymphoproliferation (LPA) against AT-2 inactivated HIV-1 antigen measured by the [^{3}H]-thymidine uptake assay (cut-off ≥ 6 SI) in HIVIS03 vaccinees at baseline, two weeks after three HIV-DNA vaccinations, two weeks after the first HIV-MVA boost and two weeks after the second HIV-MVA boost.

2 CD4$^+$ T cell response in responders to Gag I ($n = 2$) was 0.19% (0.06–0.32%), to Gag II ($n = 10$) 0.15% (0.09–0.83%) and to Gag WR ($n = 15$) 0.15% (0.07–3.67%). The median (range) IFN-γ/IL-2 CD8$^+$ T cell response in responders to Gag I ($n = 9$) was 0.18% (0.1–0.47%), to Gag II ($n = 9$) 0.30% (0.14–0.92%) and to Gag WR ($n = 10$) 0.24% (0.15–1.15%). The magnitude of the Gag-specific response (median 0.25%) was significantly higher in the CD8$^+$ T cell compartment than in the CD4$^+$ T cell compartment (median 0.15%) ($p < 0.05$). One of 12 placebo recipients had CD8$^+$ T cell responses to Gag I and Gag WR.

3.3.3. LPA responses

HIV-specific T cell responses were also assessed using the LPA. Two weeks after the third HIV-DNA injection, 11/16 (69%) id and 8/15 im (53%) evaluable vaccinees had LPA responses. Two weeks after the first and the second HIV-MVA boost, respectively, all the 32 and all the 25 evaluable vaccinated volunteers had positive LPA responses (Fig. 3). Reactivity did not differ significantly between the id and the im groups. However, LPA responses were significantly higher two weeks after the first HIV-MVA boost compared to two weeks after the second HIV-MVA boost (median SI 196 vs 99; $p = 0.048$). There was a significant correlation ($r^2 = 0.414$, $p < 0.001$) between ELISpot and LPA responses after the first HIV-MVA boost (Fig. 4). No placebo recipient had a positive LPA response two weeks after the fifth placebo injection, which corresponded in time to the second HIV-MVA immunization in the vaccinees. However, two placebo recipients had weak positive LPA responses (SI = 12 and 30) two weeks after the fourth placebo injection.

3.4. Antibody responses

3.4.1. Binding antibodies

Antibodies to native gp160 were demonstrated in 7 of 33 (21%) evaluable vaccinees after the first HIV-MVA boost, and in 26 of 29 (90%) after the second boost. Anti-gp160 antibody titres after the second HIV-MVA boost did not differ significantly between id primed (median 800, range 200–3200) and im primed vaccinees (median 800, range 200–6400). None of the vaccinees or placebo recipients were positive in diagnostic HIV serological assays after the HIV-DNA immunizations or after the first HIV-MVA boost. After two HIV-MVA boosts, all 30 vaccinees (100%) were reactive in all three diagnostic HIV assays.

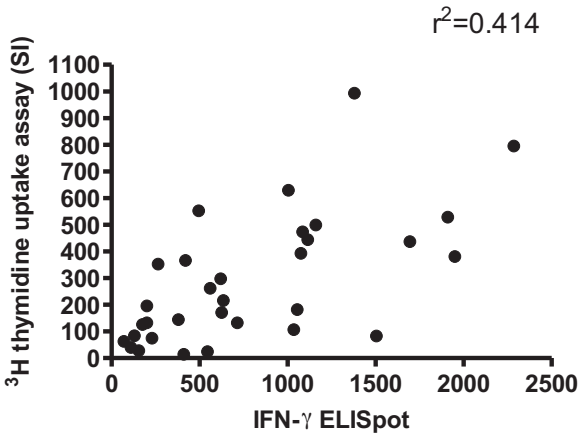

Fig. 4. Correlation between Gag WR reactivity in IFN-γ ELISpot and HIV CM responses in LPA in 50 HIVIS03 volunteers two weeks after the first HIV-MVA immunization ($r^2 = 0.414$, $p < 0.001$).

3.4.2. Neutralizing antibodies

Table 5 summarizes the NAb response rates in the 29 vaccinees primed with HIV-DNA id or im and tested four weeks after the second HIV-MVA boost. There was no demonstrable neutralizing activity in the TZM-bl pseudovirus assay using CM235 clade CRF01_AE, GS015 clade C and BaL clade B pseudoviruses. In contrast, a high antibody response rate was demonstrated using the PBMC assay. The response rates were higher against the CM235 clade CRF01_AE virus (overall 24/29, 83%) and the SF162 clade B virus (21/29, 72%) as compared to the BaL clade B virus (9/29, 31%). The response rate after the second HIV-MVA boost was not significantly different between id vs im HIV-DNA primed vaccinees ($p = 0.43$ by Fisher Exact test).

4. Discussion

This phase I/II trial primarily aimed to determine the safety and immunogenicity of a multigene, multiclade HIV-DNA prime MVA boost regimen that focused on modes of DNA vaccine delivery, besides building capacity at Tanzanian institutions for future HIV vaccine trials. The trial is an expansion of our previous HIVIS01/02 phase I trial of the same vaccine constructs in Sweden [17]. The current trial confirmed that the immunogens and routes of HIV-DNA administration with a needle-free injection device (the Biojector) followed by HIV-MVA boost administered by a syringe and needle im were well tolerated and highly immunogenic. It also demonstrated the superiority of priming id with 1 mg of HIV-DNA over

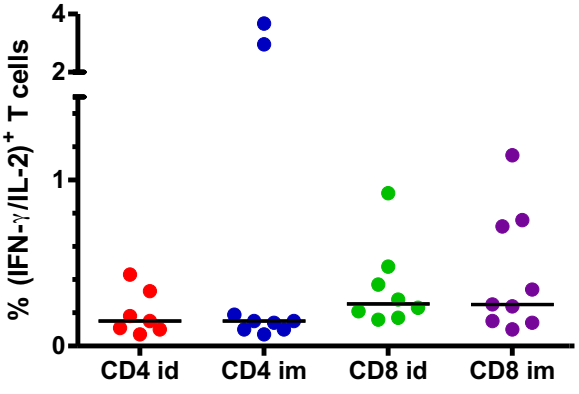

Fig. 5. Magnitude of HIV-specific CD4$^+$ and CD8$^+$ T cell responses to Gag assessed by a 4-colour IFN-γ/IL-2 ICS assay four weeks after the second HIV-MVA boost in samples from vaccinees primed with HIV-DNA id or im.

Table 5
Neutralizing antibody response rates in the 29 vaccinees primed with HIV-DNA id or im and tested four weeks after the second HIV-MVA boost.

Assay	Virus	Clade	Serum dilution	Number of positive/number tested (%)		*p* value
				id	im	
Pseudovirus/TZM-bl cells	BaL	B	1:20	0/16 (0)	0/13 (0)	
Pseudovirus/TZM-bl cells	GS015	C	1:20	0/16 (0)	0/13 (0)	
Pseudovirus/TZM-bl cells	CM235	CRF01_AE	1:20	0/16 (0)	0/13 (0)	
IMC/PBMC	BaL	B	1:60	4/16 (25)	5/13 (38)	0.43
IMC/PBMC	SF162	B	1:60	13/16 (81)	8/13 (62)	0.41
IMC/PBMC	CM235	CRF01_AE	1:60	14/16 (88)	10/13 (77)	0.63

priming im with 3.8 mg (for a total of 3 vs 11.4 mg) with the needle-free injection device. Antibodies detectable in routine serological assays were induced in all vaccine recipients and anti-Env gp 160 antibodies in 90% after the second HIV-MVA boost. Furthermore, a high neutralizing antibody response rate was demonstrated using a PBMC assay.

The IFN-γ ELISpot responses after three HIV-DNA priming injections were weak and only directed to Gag peptides. Similarly very low responses to three DNA injections by ADVAX alone at a similar concentration im has been observed [20]. However, a pronounced IFN-γ ELISpot response was seen in all volunteers in the present trial after the first HIV-MVA boost. Furthermore, there was a trend to higher responses to several Env peptide pools in those primed with HIV-DNA id, which reached significance for the Env pool that matched the boosting immunogen. Since the peptide pools used were only partially matched to the immunogen, reactivity to additional HIV-specific epitopes might have been induced as well.

A broad HIV vaccine-induced reactivity is considered desirable. In the IFN-γ ELISpot assay, all the immunized volunteers reacted with at least one of the Gag peptide pools; and in addition, 31 of 35 (89%) reacted with at least one Env pool, indicating that this heterologous immunization induced a broad and balanced immune response. Furthermore, ICS for Gag specific IFN-γ/IL-2 production showed both $CD8^+$ and $CD4^+$ T-cell responses.

The previous phase I study in Sweden suggested that age over 40 years might be associated with lower vaccine-induced responses [17]. The HIV-1 specific response rate was the same among those with prior immunity to vaccinia as among those not previously small pox vaccinated, although the magnitude of the responses was lower. However, the correlation between age and prior vaccinia vaccination complicated the interpretation [48]. In this study, we aimed to recruit only those under 40 years of age. Even so, we found a negative coefficient of correlation, $r = -0.41$, between increasing age and the induced HIV specific immune response, but the response rate was not lower among those with presence of a vaccinia scar.

The same HIV-MVA vaccine construct as was used in the HIVIS trials in Sweden and Tanzania has been evaluated in a phase I safety and immunogenicity trial that included three immunizations at months 0, 1 and 3, of healthy volunteers in the USA and Thailand [22]. The HIV-MVA vaccine was well tolerated and elicited both cell-mediated and humoral immune responses in a high proportion of vaccine recipients. The IFN-γ ELISpot response rate as well as the Env antibody response rate was 90% in the vaccinees who received the high dose of MVA-CMDR (10^8 pfu). However, the magnitude of the IFN-γ ELISpot responses, which were predominantly Env-specific, was moderate and lower than after the HIV-DNA priming and HIV-MVA boosting immunizations in the present trial. A trial of the ADMVA HIV-1 candidate vaccine showed IFN-γ ELISpot responses in 62% and antibody responses to gp120 in 77% of recipients of 3 injections of a high dose of the vaccine [23]. In a study of administration of 3 doses of the Geovax MVA/HIV62 vaccine 43% of the vaccinees had $CD4^+$ and 17% had $CD8^+$ T cell responses to IFN-γ or IL-2 demonstrable by ICS with a strong preference for Gag reactivity. Anti-Env antibodies were found in 86% of the vaccinees. However, cellular immune response rates were higher whereas antibody response rates were lower after two HIV-DNA priming immunizations and two MVA/HIV62 boosts [24]. In a study of the TBC-M4 MVA HIV-1 vaccine candidate an IFN-γ ELISpot response rate of 58–67% after the third high dose vaccination was found with a balance between Gag and Env responses. All volunteers in the high dose group developed anti-HIV antibodies to Gag and/or Env [21].

The vaccines used in the present trial differ in a number of respects from the vaccine used in the STEP trial, which studied three immunizations with Ad5 vectors carrying *gag*, *pol* and *nef* genes; and failed to show any protection from infection [27]. The current HIV-1 DNA/MVA vaccine includes Env of several subtypes and potent responses were noted against the immunogens. The Gag responses were robust and broad and included $CD8^+$ T cell responses. Furthermore, the effect of previous vector immunity was limited. The present vaccine trial also differs in several aspects from a number of completed and on-going trials of the HIV-DNA prime and pox virus boost concept [13–16,24,25]. In most trials the HIV-antigen for boosting is homologous to the priming and usually consists of antigen of a single clade. None use id priming. In this trial we have used a multiclade prime (Env A, B and C and Gag A and B) followed by a heterologous boost (Env E and Gag A) in an effort to direct the immune system against common epitopes that might be less immunogenic, and increase the likelihood that individuals with different HLA types will recognize some epitopes in the vaccine. Strong and broad cell-mediated immune responses predominantly directed against Env, have been demonstrated after HIV-DNA priming and NYVAC boosting vaccination [15]. Antibodies against gp140 have also been observed in a high proportion of vaccinees after repeated boosting with the pox virus vector [15]. There is thus an urgent need to establish the strengths and weaknesses of the various concepts in order to proceed to efficacy studies as soon as possible.

Several groups, in addition to ours, have evaluated the Biojector and included it in ongoing studies [9,11,17,49,50]. Most of these groups are using the Biojector for im injections [9,11,49]. We could show that using it for id priming not only enhanced certain aspects of the immune response after boosting, but also allowed a reduction of the HIV-DNA dose commonly used im. It has also been shown that id injections will target antigen-presenting cells in the skin [51]. Five id injections given at the same time were well tolerated in this and in the previous study [17], however this is impractical and a follow-up trial in Tanzania, TaMoVac I (Tanzania and Mozambique HIV Vaccine Programme), is currently testing whether a higher HIV-DNA concentration will permit priming with only two id injections per immunization.

Although the DNA prime/poxvirus boost vaccine regimen was designed to induce cell-mediated immune responses it is remarkable that after the second HIV-MVA boost all vaccinees developed antibodies demonstrable by routine diagnostic HIV serological tests, 90% displayed antibodies to Env gp160 and a high proportion had Nab with a similar response rate in id and im HIV-DNA-primed

vaccinees. HIV-DNA prime/pox virus boost can thus be used as a platform for antibody induction.

The functional NAb responses elicited by DNA priming and MVA boosting in this trial were very intriguing in that the two different assays employed showed completely different results. While the TZM-bl neutralization assay showed no NAb activity at all, the results from the PBMC assay were positive, with up to an 83% positive response rate against the vaccine homologous IMC from clade CRF01_AE. The observation that antibodies can be inhibitory using a PBMC target cell assay, but non-functional in a cell line based pseudovirus assay has been reported in previous studies [52–55], but has not been reported to date using human vaccine sera. This result might be explained by the fact that the TZM-bl pseudovirus assay is a single round assay and primarily assesses inhibition of virus binding and entry. It is possible that some antibody subpopulations may not be detected through the use of a single round assay focusing only on virus entry, as compared to a PBMC assay incorporating multiple rounds of infection, and thus capturing potential inhibition at all stages of the virus life cycle. However, the mechanism for the inhibitory activity in the PBMC assay employed in these studies remains to be defined and is currently under investigation.

HIVIS03 is the first HIV vaccine clinical trial to be conducted in Dar es Salaam and only the second in Tanzania. It has relied on previous long-term investments through support from the Swedish Development Agency, (Sida), for HIV-related studies, including cohort development [32] and clinical and laboratory capacity-building, whereby researchers have been able to perform all clinical and laboratory parts of the trial in Tanzania, except Nab testing.

Volunteers from the Police Force were chosen for this study because this highly organized institution favoured the probability of long-term follow-up [56]. Their participation was purely altruistic and voluntary. The attitudes of family and significant others proved very important and led to a number of post-randomization defaults, all prior to the first injection. Those who accepted the first HIV-DNA/placebo immunization displayed good adherence to the visit schedule until after completion of the third HIV-DNA/placebo dose, but subsequently a number of volunteers dropped out before the first and second HIV-MVA/placebo immunization due to various social and medical reasons. Anaemia, especially in potential female volunteers, proved to be an important limiting factor in recruitment. The vaccines did not cause the discontinuation of any of individual who did not receive the full vaccination schedule. However, the relatively high dropout rate underscored the need for stringent inclusion criteria and attention to the social and community stresses and concerns in a developing nation. A number of issues related to our trial need to be addressed. The current id immunization schedule needs to be simplified; this is addressed in TaMoVac-I. The lack of reactivity to the RT/pol component needs to be considered. The current vaccine composition needs to be enhanced to induce better B-cell responses.

In conclusion, this HIV-1 DNA prime/MVA boost approach is safe and highly immunogenic among healthy volunteers in Tanzania. The low dose, id, multigene, multiclade HIV-DNA elicited higher and broader cellular immune responses to Env compared to a higher dose given im after boosting with MVA containing heterologous HIV genes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2011.08.001.

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