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Safety, Immunogenicity and Efficacy of Modified Vaccinia Ankara (MVA) Against Dryvax® Challenge in Vaccinia-Naïve and Vaccinia-Immune Individuals

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Abstract

Modified vaccinia Ankara (MVA) was evaluated as an alternative to Dryvax® in vaccinia-naïve and immune adult volunteers. Subjects received intramuscular MVA or placebo followed by Dryvax® challenge at 3 months. Two or more doses of MVA prior to Dryvax® reduced severity of lesion formation, decreased magnitude and duration of viral shedding, and augmented post-Dryvax® vaccinia-specific CD8+ T cell responses and extracellular enveloped virus protein-specific antibody responses. MVA vaccination is safe and immunogenic and improves the safety and immunogenicity of subsequent Dryvax® vaccination supporting the potential for using MVA as a vaccine in the general population to improve immunity to orthopoxviruses.

Keywords

smallpox; orthopoxvirus; vaccine

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Author Contributions

BSG, LHM, and MEE developed the protocol for the study. BSG, LHM, JP, IG, SR, BL and the VRC 201/203 study team enrolled subjects, completed study visits, evaluated adverse events, and/or collected clinical trial data. ZM and LG performed statistical analysis and managed the weekly safety reports. Additional statistical analysis was performed by LY. MR, RB, RAK, ZM, LY and BSG analyzed the immunogenicity data. BSG, JP, LHM, MEE, ZM, and LY analyzed the clinical data. RAK, MR, and RB performed validated T cell assays. JP performed kinetic ELISA assays. BSG, JP, MEE, and LHM wrote the first draft. All authors contributed to writing the final manuscript.

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Competing Interests

No authors have competing interests.

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INTRODUCTION

Twenty-six years after the World Health Organization's declaration of global eradication of smallpox, the concern for its potential use as an agent of bioterrorism remains. With variola major mortality rate of 30% among unvaccinated individuals and more than 100 million deaths attributed to smallpox in the 20th century alone, smallpox is historically one of humankind's most feared diseases[1]. Eradication of smallpox, an infectious disease caused by the orthopoxvirus variola, was achieved through surveillance and vaccination with a highly effective live vaccinia virus vaccine. The last known naturally occurring case of smallpox was in 1977 in Somalia [2]. In the United States, the last case of smallpox occurred in 1949 and routine vaccination of the general population ceased in 1972 [3].

In spite of the efficacy of traditional replication-competent vaccines, safer options for immunoprophylaxis are being sought because of their associated rare but serious side effects. Complication rates could be even higher today because of the growing number of people in whom the vaccine is contraindicated including individuals with atopic dermatitis or those who are immunocompromised [4].

Modified vaccinia Ankara (MVA) is an alternative vaccine candidate because it is a highly attenuated vaccinia virus which has limited ability to replicate in mammalian cell lines and has previously been used in animal and human studies. MVA was derived from the CVA Dermovaccinia strain. Genes encoding proteins with immunomodulatory functions and host range determinants were lost during serial passage through chick embryo fibroblasts leading to attenuation of replication and virulence [5]. On the 516th passage it was renamed MVA [5]. More than 15% of the original vaccinia genome has been lost [6], but most genes encoding structural proteins were retained, suggesting that key antigenic determinants have been preserved. Thus, MVA has the capacity to be both safer and more immunogenic than the replication competent vaccinia currently licensed for smallpox vaccination.

Human MVA studies were conducted in more than 120,000 people in Germany in the 1970s. MVA was well tolerated and safe even in children and the elderly and resulted in an attenuated response to the live Elstree vaccinia strain administered weeks to months later. [5] Recently, MVA safety and immunogenicity were evaluated at different doses and different routes of administration in vaccinia-naïve and vaccinia-immune volunteers. MVA was safe, but the immune response was dose-dependent. This study did not include a challenge with live vaccinia vaccine following MVA administration.[7]

Animal studies have shown that MVA is both safe and immunogenic in healthy and immunosuppressed murine and macaque models. [8,9] [10] MVA immunization has also proven protective in mice challenged with a recombinant vaccinia virus with enhanced virulence due to mIL-4 transgene expression [11].

With the emerging threat of bioterrorism, the need for a safer smallpox vaccine has assumed new importance. A safer vaccine, however, has other potential utility. It could replace the current practice of Dryvax[®] vaccination in laboratory and healthcare personnel working with poxviruses. In addition, it could afford protection against zoonotic infections caused by orthopoxviruses, such as monkeypox. Therefore, we conducted clinical trials in vaccinia-naïve and vaccinia-immune subjects to evaluate the safety and immunogenicity of MVA.

METHODS

Vaccine

The MVA vaccine used in this study was prepared by Therion Biologics Corporation (Cambridge, MA) and designated TBC-MVA. TBC-MVA is a plaque-purified isolate from an original MVA seed virus provided by Dr. Anton Mayr. Study vials contained 300 μ L TBC-MVA in PBS with 10% glycerol. The selected dose for this study as measured by the manufacturer in a validated assay was 10^8 PFU. However, when measured by an independent assay, relative to the stock of MVA used to protect macaques from monkeypox [8] the delivered dose was $\sim 10^6$ pfu, henceforth referred to as an adjusted dose. The difference in titer was not related to product stability, but to the different assay method used to measure the number of plaques. Therion Biologics Corporation using a different cell line and different staining techniques consistently obtained the same titer for the vaccine product throughout the duration of the trial and beyond. The placebo for TBC-MVA was phosphate buffered saline (PBS).

Dryvax[®] and the diluent were provided by the Centers for Disease Control and Prevention (CDC). The vaccine was prepared as a lyophilized preparation of live vaccinia virus from calf lymph. The reconstituted vaccine contained approximately 10^8 PFU per mL [12]. The diluent contained 50% glycerin, 0.25% phenol in Sterile Water for Injection, USP (Chesapeake Biologics Lab, Inc).

Study Design and Subjects

The Phase I/Ib studies VRC 201 and VRC 203 were conducted as single site studies at the National Institutes of Health (NIH) by the Vaccine Research Center (VRC). They were randomized, placebo-controlled, double-blinded studies of MVA in vaccinia-naïve and vaccinia-immune subjects, respectively. The randomization sequence was obtained by computer-generated random numbers and provided to the pharmacist by the statistician. The subject, the clinical staff, and the principal investigator were blinded to treatment allocation. The randomization code was kept by the pharmacist who was primarily responsible for drug dispensing.

The primary objectives of both studies were to evaluate the safety of MVA administered by intramuscular (IM) injection on single and multidose schedules, and to identify a schedule of MVA that provided clinical evidence of protection against a 12-week vaccinia (Dryvax[®]) challenge. The secondary objectives were to compare the immunogenicity of Dryvax[®] and MVA as measured by vaccinia-specific neutralizing antibody and T cell responses. A dose of MVA was chosen to be comparable to the delivered dose of vaccinia in the Dryvax[®] inoculation, which was known to be immunogenic.

VRC 201 enrolled healthy volunteers, age 18–33 years, without prior vaccination with any vaccinia product. For VRC 203, study eligibility was based on the requirement for healthy volunteers, born no later than 1979 but less than 61 years of age who had been previously immunized with vaccinia more than 10 years ago with evidence of a vaccinia scar. Volunteers in both protocols had to be in good general health as determined by medical history, physical examination, and laboratory tests. Volunteers were excluded from either protocol if they had any contraindication to receive Dryvax[®] vaccination or a history of heart disease or 3 or more risk factors for coronary heart disease (Table 1).

Vaccinia-naïve subjects were randomized to receive one dose of Dryvax[®] or a 0.5 mL IM injection of TBC-MVA or placebo on a 1, 2, or 3 dose schedule (Figure 1a). Vaccinia-immune subjects were randomized to receive 1 or 2 doses of TBC-MVA or placebo (Figure 1b). Twelve weeks following primary vaccination series all participants were scheduled to receive

Dryvax® challenge administered by a bifurcated needle puncture to deliver 15 strokes within a 5 mm diameter area.

Following Dryvax® administration, a protective adhesive, air-permeable Opsite® dressing was applied. As previously described, gauze was applied around the lesion with a window cut out immediately over the lesion prior to the Opsite® once a vesicle or pustule formed [13]. After the lesion crusted and was without drainage, gauze dressing alone was used until scab separation.

Subjects returned to the clinic every 3–4 days until a dry scab had formed. The lesion was photographed at each follow-up visit. If a lesion or scab was present, the challenge site was swabbed with a sterile cotton swab and inoculated into 1 mL of Eagle's minimum essential medium supplemented with 10% fetal calf serum, and stored at –80° C as previously described for semi-quantitative plaque assays [13].

Safety Evaluation

Safety evaluations included monitoring of physical and laboratory assessments by clinicians. Local reactogenicity was assessed by the clinical staff at the time of visits. A group of independent study monitors comprised of three senior clinicians (Lynn Stansbury, Joel Breman, and John Bennett) with experience evaluating poxvirus lesions was available for consultation and safety reviews. Subjects self-assessed for local and systemic symptoms by diary cards recorded for 7 days after each MVA immunization and until healing after Dryvax® immunization, which was typically 3 weeks. Mild symptoms were transient or minimal without limitation in activity. Moderate symptoms were defined as producing a reduction in normal activity with minimal medication used as needed to relieve discomfort. Severe symptoms resulted in marked reduction of normal activity with hospitalization and/or medical intervention.

Adverse events were assessed for severity by a pre-approved table (Table for Grading Severity of Adult Adverse Experiences for Vaccine & Prevention Research Programs, published by NIAID Division of AIDS in 2002, see supplemental information) and graded on a 0–5 point scale and coded with the Medical Dictionary for Regulatory Activities (MedDRA). As the studies were nearing completion, cardiac evaluation including electrocardiogram, creatine kinase (CK, CK-MB, and troponin I), was added to the protocol at the request of the Food and Drug Administration (FDA). Initially, participants were followed for 24 weeks after the last MVA/placebo dose. Subjects who had not completed vaccinations at the time the FDA requested further cardiac assessment were asked to return for cardiac evaluation 24 weeks after Dryvax® vaccination. Cardiac evaluation performed 24 weeks after Dryvax® included electrocardiogram (EKG) and cardiac enzymes (CK, CK-MB, Troponin I). In addition, 14 subjects were evaluated by EKG and cardiac enzymes at 7 and 28 days following MVA vaccination and 10–12 and 20–22 days following Dryvax® vaccination.

Take Category

The take category was assessed during the period of lesion formation and recorded at the last follow-up visit. Clinical protection was judged by the characteristics of a take site reaction and assigned a take category based on a modification of the CDC guidelines (Figure 2a). CDC guidelines which define “takes” and “non-takes” recommend evaluation be done within 6–8 days following vaccination for best reliability [14]. In these studies each subject was evaluated between days 6–8 as recommended. In addition, the inoculation site was evaluated more frequently, every 3–4 days. Adhering to this schedule of more frequent evaluation of the inoculation site allowed for the comparison of take responses to the typical course of smallpox

vaccination with traditional vaccines and permitted the further categorization of the reaction beyond just take or no take.

A take category of 0 indicated no take skin reaction, a take category of 1 corresponded to a modified take skin reaction without vesicle, a take category of 2 represented a modified take skin reaction with vesicle and a take category of 3 reflected a primary take skin reaction. A primary take reaction was defined by reddening of the inoculation site 3–4 days after vaccination followed by vesicle formation with umbilication and pustular formation by the 7th to 11th day after vaccination. Lesion crusting occurred between the 2nd and 3rd week, and scab separation by the end of the 3rd or 4th week, followed by permanent scarring. Take category reactions of <3 in vaccinia-naïve and <2 in vaccinia-immune volunteers were considered evidence of clinical protection.

Plaque Assay

If a lesion or scab was present, the injection site was swabbed with a sterile cotton swab and inoculated into 1.0 mL of Eagle's minimum essential medium supplemented with 10% fetal calf serum. These samples were stored at -80°C as previously described [13]. BSC-40 cells, obtained from Dr. Shiu-Lok Hu (University of Washington, Seattle, WA) and originally derived from BSC-1 were used for plaque assay. Cells were grown in minimum essential medium (Atlanta Biologicals, Lawrenceville, GA) supplemented with 10% fetal bovine serum (10% MEM). Samples were quick-thawed at 37°C and sonicated in a water bath for 15 seconds. Serial 10-fold dilutions of samples were made and 50 μL of sample dilution were added to each well of subconfluent BSC40 monolayers in triplicate in 12-well plates. After one hour incubation on platform rocker, plates were covered with 0.75% methylcellulose in 10% MEM and incubated at 37°C . After two days incubation, plates were fixed with 10% formalin solution, stained with Gentian violet solution (Fisher Scientific company, Newark, DE), and the number of plaques were counted under the dissecting microscope. The viral titer was calculated using the formula of average number of plaques/well (at the dilution with at least 5 plaques per well) $\times 20 \times$ dilution. Data are represented as $\log_{10}$ PFU/ml of sample.

Beta-galactosidase Neutralization Assay

Serum was heat-inactivated at 56°C for 30 minutes, then stored at -80°C until use. Two-fold serum dilutions were tested in quadruplicate in parallel with positive (VIGIV FDA Standard, NIH preparation) and negative (Naïve vaccinia serum, SeraCare Life Sciences, Oceanside, CA) control sera. Recombinant vaccinia virus expressing β -galactosidase under the control of a synthetic early/late promoter (vSC56) was prepared as described in BSC-1 cells (ATCC Manassas, VA) [1] and used for neutralization assays in HeLa cells (ATCC CCL-2) using a β -galactosidase end point as described [2]. β -galactosidase standards (Roche Diagnostics) and samples were probed with β -galactosidase substrate (CPRG, Roche Molecular, IN) and the reaction was stopped after 30 minutes with 1 mol $\text{Na}_2\text{CO}_3/\text{L}$ ("Stop" buffer, Roche Molecular, IN). Plates were read immediately using a VersaMax plate reader (Molecular Devices, Sunnyvale, CA) at OD_{575} and analyzed with SoftMax software (Molecular Devices, Sunnyvale, CA) to determine the serum dilution giving 50% neutralization. The assay parameters were validated and quality assurance was monitored by daily assays on control sera. Assays were repeated if the standard deviation of replicate wells exceeded 15% of the mean, and if the virus only control wells were outside the range of 2200 to 6300 mU/mL.

Kinetic ELISA Assay

Protein specific antibody was determined by kinetic ELISA in a procedure modified from Boyce et al[15]. Two IMV purified proteins (A27L and L1R) and two EEV purified proteins (A33R and B5R) produced from baculovirus were obtained from Dr. Gary H. Cohen at the University of Pennsylvania. These antigens were diluted in carbonate buffer (pH 9.6) and

coated onto 96-well flat bottom ELISA plates (Nunc, Rochester, NY) at a concentration of 80 ng/well. Duplicate wells were coated with 100 μ L of the diluted antigen and 100 μ L of carbonate buffer was added to an additional well for each sample to be tested. Plates were coated overnight at 4° C. Nonspecific adsorption was prevented with 200 μ L/well of blocking buffer (2% nonfat dry milk) for 1 hour at 37° C. Plates were washed four times on an automated plate washer (Bio-Tek Instruments, Winooski, VT) with wash buffer (0.02% Tween-20 in PBS).

One hundred μ L of serial dilutions of VIG (obtained from the FDA) as a positive control or 100 μ L of diluted test sera (1:100 in blocking buffer) were added to each well in triplicate (two coated wells and one uncoated well). Plates were incubated for one hour at 37° C, washed four times, and incubated for 1 hour at 37° C with 1:10,000 dilution of HRP-conjugated goat antihuman IgG + IgM antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Plates were washed with wash buffer four times followed by distilled water. One hundred μ L of Super AquaBlue ELISA substrate (eBioscience) was added to each well and plates were read immediately using a Dynex Technologies microplate reader (Chantilly, VA). The rate of color change in mOD/min was read at a wavelength of 405 nm every 9 seconds for 5 minutes with the plates shaken before each measurement. The mean mOD/min reading of duplicate wells was calculated, and the background mOD/min was subtracted from the corresponding well.

Intracellular Cytokine Staining

Peripheral blood mononuclear cells (PBMC) were prepared by standard Ficoll-Hypaque density gradient centrifugation (Pharmacia; Uppsala, Sweden). PBMC were frozen in heat-inactivated fetal calf serum containing 10% dimethylsulfoxide in a Forma CryoMed cell freezer (Marietta, OH), and stored at -140° C. All assays were performed on thawed specimens; average viability was >95%. One million PBMC in 200 μ L R-10 medium (RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin G, 100 μ g/mL streptomycin sulfate, and 1.7 mM sodium glutamate) were infected at a MOI = 0.5 with Vaccinia (TBV-Wy) and MVA (TBC-MVA) (Therion Biologics, Cambridge, MA) in separate wells of 96-well V-bottom plates. Uninfected cells were included in every experiment to control for spontaneous production of cytokine and activation of cells prior to infection.

Staphylococcal enterotoxin B (SEB, 10 μ g/mL, Sigma-Aldrich) was used as a positive control for lymphocyte activation. Cultures were incubated at 37°C in a 5% CO₂ incubator for 5 hours, after which brefeldin A (10 μ g/mL; Sigma, St. Louis, MO) was added and incubation continued for an additional 12–16 hours. Cells were permeabilized for 7 min in 200 μ L of a solution containing 67 μ L Tween20 (Sigma), 106 μ L DI water, and 27 μ L of 10x FACS-Lyse solution (Becton Dickinson Immunocytometry Systems (BDIS)) at room temperature, washed twice in cold Dulbecco's phosphate-buffered saline containing 1% fetal bovine serum and 0.02% sodium azide (FACS (fluorescence-activated cell sorting buffer), and stained for 15 min on ice with APC-conjugated mouse anti-human CD3, FITC-conjugated mouse anti-human CD8, PerCP-conjugated mouse anti-human CD4, and a mixture of PE-conjugated mouse anti-human IFN- γ and IL-2 (Becton Dickinson Immunocytometry Systems, San Jose, CA). All reagents were independently titrated to determine the optimum concentration for staining. Stained cells were then immediately washed twice with cold FACS buffer. The cells were resuspended in Dulbecco's phosphate-buffered saline containing 1% paraformaldehyde (Electron Microscopy Systems; Fort Washington, PA) and stored at 4°C until analysis. Four color flow cytometric analysis was performed on a FACSCalibur flow cytometer (BDIS). Between 50,000 and 250,000 events were acquired, gated on small lymphocytes, and assessed for CD3, CD8, CD4, and IFN- γ /IL-2 expression. Results were analyzed using FlowJo software (Tree Star Software; Ashland, OR). The same cytokine, CD4, and CD8 gates were used for the entire trial.

Statistical Analysis

In most of the analyses, the study results are grouped as Dryvax[®] only, one dose of MVA, two doses of MVA and three doses of MVA, and two doses of Dryvax[®]. The “Dryvax[®] only” group combines the group(s) that received placebos prior to Dryvax[®] challenge and data post the first Dryvax[®] in the two dose Dryvax[®] group. Comparisons are made among the group using Fisher’s Exact test for binary outcomes (take rate, positive response rate, etc.), or Wilcoxon rank sum test for continuous outcomes (e.g. peak lesion size). Significance levels ($\alpha=0.05$) as well as confidence intervals are adjusted using Bonferroni method where multiple comparisons are made. SAS (Version 8.2; SAS Institute) and Splus (Version 6.0; Insightful) were used for all analyses. All tests are two-sided.

Positive response rates to vaccinia and MVA were used to summarize the T cell response data. The positivity criteria for the ICS data consisted of a statistical hypothesis test for a difference in the stimulated and unstimulated wells followed by the requirement of a minimal level of response. For an individual’s response to be categorized as positive, it had to be statistically significant and had to exceed the threshold for positivity. For the ICS responses, a Fisher’s exact test was applied to each antigen-specific response vs. the negative control response with a Holm adjustment for the multiple comparisons. The nominal significance level was $\alpha=0.01$.

The schedule selection was based on take rates; schedules whose true take rate lie at or above the targeted rate of 70% with at least 95% probability would be selected for further study. That is, the lower bound of the 2-sided 95% simultaneous confidence interval (Bonferroni-corrected) must exceed 70% for selection. If no schedule satisfied these criteria, the schedule with the highest observed response rate would be selected.

Neutralization antibody titers and viral shedding (lesion titers from swab) were summarized using geometric means and their 95% confidence intervals. Lesion results were presented, and pair-wise comparisons were made to compare the results at each time point using Wilcoxon Rank Sum test. Linear regression with repeated measure (PROC GLM with REPEATED in SAS) was also employed to examine the difference among the groups with repeated measures over time.

For the kinetic ELISA response data all comparisons were done using one-way Analysis of Variance to determine if differences between the groups were present; when the ANOVA indicated differences between the groups, Bonferroni-adjusted p-values were given for pair-wise comparisons. For the comparisons in 203 p-values were adjusted for 3 pair-wise comparisons; for the comparisons in 201 adjustments were based on 10 pair-wise comparisons. When differences were found between the groups, linear regressions were used to examine whether differences between groups were maintained after adjustment for Baseline mOD.

RESULTS

Between December 2002 and May 2004, a total of 76 vaccinia-naïve subjects were enrolled in VRC 201 with 64 subjects completing Dryvax[®] challenge (Figure 1a). In spite of detailed screening, one subject was later found to be seropositive for neutralizing antibody at baseline with a high suspicion of previous vaccination and thus has been excluded from all analyses. Between March 2003 and May 2004, 75 vaccinia-immune subjects were enrolled in VRC 203 with 67 subjects completing Dryvax[®] challenge (Figure 1b). In VRC 201, 68 volunteers completed the protocol with 8 early terminations. Of the 12 people not vaccinated, 2 had unrelated adverse events which prevented the administration of Dryvax[®] challenge. The other subjects were dropped from the vaccination schedule for reasons including subject withdrawal, relocation, and lost to follow-up. Initially, target enrollment was 105 vaccinia-naïve subjects and 80 vaccinia-immune subjects; however, both studies were stopped prior to achieving the

target after it was concluded by the Data and Safety Monitoring Board that the number of subjects enrolled was sufficient to answer the primary objectives of the studies, and would delay analysis.

Safety

Self-assessed local and systemic reactogenicity are shown in Table 2. Following the primary vaccination series, no subjects in the placebo or MVA vaccine groups developed a lesion at the vaccination site; however, the MVA recipients did report significantly more pain at the vaccination site (Table 2A). Following Dryvax® challenge, subjects who had received placebo during the primary vaccination series developed the most severe lesions and local symptoms (Table 2B). Vaccinia-naïve placebo recipients had significantly greater peak erythema ($p=0.0001$, Wilcoxon rank sum test), induration ($p=0.046$, Wilcoxon rank sum test), and lesion size ($p=0.0005$, Wilcoxon rank sum test) compared with MVA recipients. Vaccinia-immune placebo recipients had significantly greater peak erythema ($p=0.0001$, Wilcoxon rank sum test) and induration ($p=0.028$, Wilcoxon rank sum test) compared with MVA recipients.

No serious systemic reactions were reported. There were no episodes of myocardial infarction, myopericarditis, or cardiac chest pain. EKG monitoring of subjects was initiated near the end of the study and data was collected on 14 vaccinia-naïve and vaccinia-immune individuals. There were no cardiac events noted during this period of follow-up.

Three vaccinia-naïve volunteers, all in the placebo group, developed skin manifestations following Dryvax® that were determined to be possibly or probably related to vaccine presented with a non-specific macular rash 8 days after Dryvax. The rash was of moderate severity and began with an intensely pruritic 1 mm papule on the left wrist. By day 10 the volunteer had approximately fifteen 8 × 8 mm macules on the trunk, chest, and back. The rash resolved within 6 days. Another volunteer developed a mild dermatitis of unknown etiology that was assessed as possibly related to vaccine. Five days after Dryvax® administration, the volunteer reported pruritic red papules on the hands and feet which she attributed to a change in laundry detergent. The symptoms resolved one day later after changing detergent. A third volunteer reported eruption of three blisters on the left palm six days after receiving Dryvax®. The reaction was rated as mild and the subject denied pruritus, pain, or drainage. The blisters were covered with a bandaid and spontaneously resolved one day later.

Take Category

Vaccinia-naïve volunteers who received any dose of MVA prior to Dryvax® had attenuated lesion severity and duration as represented by a decrease in the number of primary take reactions, take category 3 (Figures 2a and 2b). Attenuated take reactions were present in 14/17 (=82.4%, 95% simultaneous CI=51%, 97%) of the one-dose MVA group, 15/15 (=100%, 95% simultaneous CI=70%, 100%) of the two-dose MVA group, 12/13 (=92.3%, 95% simultaneous CI=55%, 100%) of the three-dose MVA group, 8/8 (=100%, 95% exact CI=52%, 100%) of the two-dose Dryvax® group, and 4/20 (=20%, 95% exact CI=4%, 51%) of the Dryvax® only group. The difference between primary take reactions in the combined 2 and 3-dose MVA group (27/28) compared to the Dryvax® only (4/20) was statistically significant ($p<0.001$, Pearson's Chi-square test).

Regardless of vaccination group, no vaccinia-immune subjects developed a primary take skin reaction following Dryvax® challenge (Figure 2c). Attenuated take category reactions of <2 were present in 7/19 (=36.8%, 95% simultaneous CI=13%, 66%) of the one-dose MVA group, 7/20 (=35.0%, 95% simultaneous CI=(13%, 64%)) of the two-dose MVA group, and 3/28 (=10.7%, 95% exact CI=(2%, 32%)) of the Dryvax® only group. The vaccinia-immune MVA vaccinees tended to have less severe take reactions. Although, there was no statistically

significant difference in primary take reactions between either vaccine group compared to the placebo group, there was a significant difference in the combined vaccine groups (14/39) and the placebo group (3/28) ($p=0.02$, Pearson's Chi-square test with Monte Carlo p -value).

Vaccinia Replication

In the vaccinia-naïve groups, receiving two or three doses of MVA prior to Dryvax[®] resulted in a significant decrease in viral shedding (lesion titers) by 9–12 days (Figure 3a) when compared with the Dryvax[®] only recipients ($p=0.0026$ and 0.03 , respectively, Wilcoxon rank sum test). The longitudinal differences during the first two weeks post Dryvax[®] challenge, are also marginally significant (insignificant after adjustment for multiple comparisons) ($p=0.04$ and 0.03 , respectively, linear regression with repeated measure). In vaccinia-immune subjects receiving two doses of MVA prior to Dryvax[®] resulted in a significant decrease in viral shedding at an earlier stage, 6–8 days (Figure 3b) compared with Dryvax[®] only and one-dose MVA recipients ($p=0.0017$, Wilcoxon rank sum test). This difference is also significant when longitudinal model is applied ($p=0.003$, linear regression with repeated measure).

Antibody Responses

MVA broadly induced both antibody and T cell responses (Figures 4 and 5). Following MVA injections at this adjusted dose level of 10^6 pfu/ml, there was no appreciable increase in geometric mean vaccinia neutralizing antibody titers compared to baseline in vaccinia-naïve and prior vaccinia recipients. After Dryvax[®] challenge, MVA vaccinees developed geometric mean titers similar to the Dryvax[®] only vaccine groups (Figure 4a). For the EEV proteins B5R and A33R the vaccinia-naïve two-dose MVA group showed a greater response following Dryvax[®] challenge than either the Dryvax[®] only ($p\leq 0.03$, ANOVA) or Dryvax[®]/Dryvax[®] groups ($p\leq 0.002$, ANOVA) (Figures 5b and 5c). Post-challenge there was also a significantly greater antibody response to the EEV proteins among the three-dose MVA group compared with the Dryvax[®]/Dryvax[®] group ($p\leq 0.003$, ANOVA) (Figures 5b and 5c). Among the vaccinia-immune groups there were no statistically significant differences in the antibody responses to any of the proteins assayed (data not shown).

T Cell Responses

In vaccinia-naïve and vaccinia-immune individuals there was no significant difference in vaccinia-specific CD4⁺ responses between placebo, MVA, or Dryvax[®] recipients following primary vaccination or after Dryvax[®] challenge (Figure 4b).

In contrast, MVA induced detectable vaccinia-specific CD8⁺ CTL responses prior to challenge in more than 80% (26/31) of vaccinia-naïve subjects receiving 2 or 3 doses of MVA. After Dryvax[®] challenge, there was a significantly greater (all $p<0.05$, Holm adjusted Wilcoxon Rank Sum tests) magnitude of CD8⁺ response in 2- and 3- dose MVA recipients compared to placebo, Dryvax[®] and 1-dose MVA groups (Figures 4c and 5a). Only 4% (3/70) of vaccinia-immune subjects had detectable vaccinia-specific CD8⁺ CTL responses at baseline. After 2 doses of MVA, 85% (17/20) of subjects had detectable responses.

DISCUSSION

MVA was well tolerated among the 99 subjects who received a total of 179 injections of 10^6 pfu and no serious adverse events were attributed to MVA. Importantly, prior vaccination with MVA decreased the reactogenicity and improved the immunogenicity of Dryvax[®]. This is consistent with the limited ability of MVA to replicate in mammalian cells [16] and with the extensive evaluation of MVA in humans in the mid 20th century that reportedly demonstrated MVA to have an excellent safety profile[5].

The local and systemic reactogenicity of MVA was minimal. None of the rare idiosyncratic serious events associated with Dryvax[®] inoculation were observed in these studies after either MVA or Dryvax[®]. In particular, there was no evidence of cardiac complications that have been raised as a theoretical concern because of the myopericarditis occasionally seen after Dryvax[®] immunization [17]. The safety record of MVA should allow immunization of expanded populations of subjects including groups excluded from Dryvax[®] inoculation. Larger trials should be performed including subjects at the extremes of age and those with eczema, atopy, or immunodeficiency in addition to larger numbers of healthy adults.

The efficacy of MVA was tested in this study by Dryvax[®] challenge at 3 months after the vaccine regimen. Vaccinia-naïve volunteers who received Dryvax[®] alone had responses typical of non-immune persons with scab formation by the end of the second week and scab separation by the end of the third week according to CDC guidelines [18,19]. In a prior study, scab separation occurred closer to the end of the 4th week[20]. The difference in duration of lesion healing may have been affected by the dressing technique. In our subjects a sterile transparent dressing was initially applied and replaced by gauze pad under an occlusive dressing when the lesion became purulent. In the Lancet paper a simple non-occlusive dressing was applied.

Prior MVA immunization significantly reduced lesion severity and systemic reactogenicity induced by the Dryvax[®] challenge in vaccinia-naïve subjects, and two or three doses of MVA reduced the duration of vaccinia replication after Dryvax[®] challenge. These effects were less dramatic in vaccinia-immune subjects because of the significant preexisting residual immunity from prior Dryvax[®] vaccination. However, two doses of MVA, even in vaccinia-immune subjects, resulted in diminished vaccinia replication following Dryvax[®] challenge. Therefore, a 2-dose regimen is recommended for future studies that evaluate duration of protective immunity.

It was shown in reports from the 1970s that prior MVA immunization could reduce the reactogenicity of vaccinia inoculation. Those studies typically combined MVA with live vaccinia virus administered 1–2 weeks later as a proposed vaccination schedule. A theoretical concern of combining MVA with Dryvax is that diminished vaccinia-induced inflammation may reduce the immunity afforded by vaccinia vaccination. In the present studies, subjects were inoculated with vaccinia 3 months after completing the MVA vaccine regimen with the intent of challenging with live vaccinia virus in the memory phase of the initial MVA-induced immune response. We found in both vaccinia-naïve and vaccinia-immune subjects in these studies, two MVA doses of 10⁶ pfus (adjusted dose) induced detectable T cell responses in the large majority of individuals, and post Dryvax[®] inoculation the magnitude of vaccinia-specific CD8+ T response was significantly increased above subjects who received Dryvax[®] alone. While the neutralizing antibody titer was not different between groups, there was an increase in the response to EEV proteins in the vaccinia-naïve individuals who received MVA. The EEV virion form is believed to be a determinant of long-range spread of the virus within the host[21] and may be an important target for vaccine-induced immunity. The increased vaccinia-specific CD8+ T cell response and EEV antibody response indicates that 2 injections with this dose of MVA not only attenuates the clinical reaction to Dryvax[®] inoculation but enhances Dryvax[®] immunogenicity. A detailed characterization of the phenotype and functionality of the T cell response will be described elsewhere (Precopio et al., in submitted).

The dose of MVA may affect the antibody response to MVA and consequently the level of protection from the immediate Dryvax[®] challenge. The 10⁶ adjusted dose did not elicit an appreciable antibody response prior to challenge which allowed limited vaccinia replication to occur which may account for the higher CD8+ T cell responses post-challenge in MVA recipients. Complete protection against vaccinia would be desirable for laboratory workers

using recombinant viruses, and therefore higher doses of MVA should particularly be considered for occupational indications. However, partial immunity as noted in this study may have advantages if Dryvax[®] inoculations are needed to control a future smallpox outbreak. Since this was a schedule-finding study evaluating the number of MVA injections, future studies should include formal dose escalation using 2 MVA injections. These studies will be necessary to determine whether a higher dose of MVA will induce better antibody responses, to assess the level of protection against Dryvax[®], and to define the duration of immunity.

We were not able to precisely define an immune correlate of protection, but induction of CD8 + T cell responses post MVA immunization and the magnitude of the CD8+ T cell response post Dryvax[®] challenge were associated with reduced severity of lesion formation following Dryvax[®] inoculation. Vaccine-induced immune protection from orthopoxviruses in mice involves both antibody and CD8+ T cell responses, which are dependent on or improved by CD4+ T cell responses. Immunity is lost in murine models only when CD4+ T cells, CD8+ T cells, and antibody are all defective or depleted [8,11,22]. Antibodies have been shown to be an important factor in controlling monkeypox infection after intravenous challenge in macaques, but the relative importance of antibody versus CD8+ T cell for protection from a local inoculation was not evaluated [23]. Additional studies will be needed to establish the respective roles of CD8+ T cells and antibody in the speed of resolution of skin lesions caused by cutaneous inoculation or protection from a mucosal or aerosol challenge with orthopoxviruses.

While the efficacy of MVA in protecting against a challenge with a live virus vaccinia vaccination was tested in these trials, the efficacy of MVA against smallpox cannot be evaluated in humans. Therefore the FDA has adopted the “Animal Rule” which allows for potential licensure of products when efficacy testing is not possible in humans. This process involves extensive efficacy testing in well characterized animal models and defining correlates of protection that can be used as endpoints for evaluating the product in humans [24]. As an adjunct to the “Animal Rule” approach, consideration should be given for testing MVA as a vaccine against monkeypox which is still endemic in parts of equatorial Africa. During the 2003 outbreak of human monkeypox in the Republic of Congo, 11 confirmed and probable monkeypox cases were observed along with human-to-human transmission of disease. Although no healthcare workers were infected during this outbreak, there was hospital-associated transmission of disease, indicative of the possibility of larger hospital-associated monkeypox outbreaks in the future which would leave healthcare workers and their families particularly vulnerable [25]. Similar examples of extended serial human-to-human transmission have been demonstrated in Democratic Republic of Congo (personal communication, J.J. Muyembe.) MVA has an excellent safety profile and is replication deficient; it would be expected to be safe in populations where there is a high prevalence of HIV or other conditions that would require pre-screening for Dryvax[®] inoculation. Evaluating MVA immunization in a setting with a high incidence of monkeypox would provide additional guidance for how to use MVA either alone or in combination with Dryvax[®] for protection against orthopoxviruses.

Our data support further evaluation of immunizing the general population with 2 doses of MVA as a safe way to provide a platform of orthopoxvirus immunity. MVA should also be considered as a method of protecting the growing number of vaccinia-naïve laboratory workers from recombinant orthopoxviruses in biomedical research. Whether MVA immunization alone would be sufficient to protect against variola as it does against monkeypox or variola in macaques would not be known until faced with a crisis. Therefore, emergency plans should also be established for Dryvax[®] immunization of those persons without medical contraindications in the event of a smallpox outbreak. Our data suggest that prior MVA immunization will make vaccination with Dryvax[®] safer and more immunogenic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Darling RG, Catlett CL, Huebner KD, Jarrett DG. Threats in bioterrorism. I: CDC category A agents. *Emerg Med Clin North Am* 2002;20(2):273–309. [PubMed: 12120480]
2. Henderson DA, Inglesby TV, Bartlett JG, Ascher MS, Eitzen E, Jahrling PB, et al. Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *Jama* 1999;281(22):2127–37. [PubMed: 10367824]
3. Lane JM, Goldstein J. Evaluation of 21st-century risks of smallpox vaccination and policy options. *Ann Intern Med* 2003;138(6):488–93. [PubMed: 12639083]
4. McCurdy LH, Larkin BD, Martin JE, Graham BS. Modified vaccinia Ankara: potential as an alternative smallpox vaccine. *Clin Infect Dis* 2004;38(12):1749–53. [PubMed: 15227622]
5. Stickl H, Hochstein-Mintzel V, Mayr A, Huber HC, Schafer H, Holzner A. MVA vaccination against smallpox: clinical tests with an attenuated live vaccinia virus strain (MVA) (author's transl). *Dtsch Med Wochenschr* 1974;99(47):2386–92. [PubMed: 4426258]
6. Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *J Gen Virol* 1991;72 (Pt 5):1031–8. [PubMed: 2033387]
7. Vollmar J, Arndtz N, Eckl KM, Thomsen T, Petzold B, Mateo L, et al. Safety and immunogenicity of IMVAMUNE, a promising candidate as a third generation smallpox vaccine. *Vaccine* 2006;24(12):2065–70. [PubMed: 16337719]
8. Earl PL, Americo JL, Wyatt LS, Eller LA, Whitbeck JC, Cohen GH, et al. Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature* 2004;428(6979):182–5. [PubMed: 15014500]
9. Stittelaar KJ, van Amerongen G, Kondova I, Kuiken T, van Lavieren RF, Pistor FH, et al. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J Virol* 2005;79(12):7845–51. [PubMed: 15919938]
10. Stittelaar KJ, Kuiken T, de Swart RL, van Amerongen G, Vos HW, Niesters HG, et al. Safety of modified vaccinia virus Ankara (MVA) in immune-suppressed macaques. *Vaccine* 2001;19(27):3700–9. [PubMed: 11395204]
11. McCurdy LH, Rutigliano JA, Johnson TR, Chen M, Graham BS. Modified vaccinia virus Ankara immunization protects against lethal challenge with recombinant vaccinia virus expressing murine interleukin-4. *J Virol* 2004;78(22):12471–9. [PubMed: 15507634]
12. CDC, Guide B. Vaccination Guidelines for State and Local Health Agencies.
13. Graham BS, Belshe RB, Clements ML, Dolin R, Corey L, Wright PF, et al. Vaccination of vaccinia-naïve adults with human immunodeficiency virus type 1 gp160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. The AIDS Vaccine Clinical Trials Network. *J Infect Dis* 1992;166(2):244–52. [PubMed: 1353102]
14. <http://www.bt.cdc.gov/agent/smallpox/vaccination/takes-non-takes.asp>

15. Boyce TG, Gruber WC, Coleman-Dockery SD, Sannella EC, Reed GW, Wolff M, et al. Mucosal immune response to trivalent live attenuated intranasal influenza vaccine in children. *Vaccine* 1999;18(1–2):82–8. [PubMed: 10501238]
16. Wyatt LS, Earl PL, Eller LA, Moss B. Highly attenuated smallpox vaccine protects mice with and without immune deficiencies against pathogenic vaccinia virus challenge. *Proc Natl Acad Sci U S A* 2004;101(13):4590–5. [PubMed: 15070762]
17. Arness MK, Eckart RE, Love SS, Atwood JE, Wells TS, Engler RJ, et al. Myopericarditis following smallpox vaccination. *Am J Epidemiol* 2004;160(7):642–51. [PubMed: 15383408]
18. <http://www.bt.cdc.gov/agent/smallpox/training/overview/pdf/vaccineoverview.pdf>
19. <http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/normal.html>
20. Greenberg RN, Kennedy JS, Clanton DJ, Plummer EA, Hague L, Cruz J, et al. Safety and immunogenicity of new cell-cultured smallpox vaccine compared with calf-lymph derived vaccine: a blind, single-centre, randomised controlled trial. *Lancet* 2005;365(9457):398–409. [PubMed: 15680454][see comment]
21. Edghill-Smith Y, Venzon D, Karpova T, McNally J, Nacsa J, Tsai WP, et al. Modeling a safer smallpox vaccination regimen, for human immunodeficiency virus type 1-infected patients, in immunocompromised macaques. *Journal of Infectious Diseases* 2003;188(8):1181–91. [PubMed: 14551889]
22. Belyakov IM, Earl P, Dzutsev A, Kuznetsov VA, Lemon M, Wyatt LS, et al. Shared modes of protection against poxvirus infection by attenuated and conventional smallpox vaccine viruses. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(16):9458–63. [PubMed: 12869693]
23. Edghill-Smith Y, Golding H, Manischewitz J, King LR, Scott D, Bray M, et al. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. *Nat Med* 2005;11(7):740–7. [PubMed: 15951823]
24. 21 CFR 314 Subpart I. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearchcfm?CFRPart=314&showFR=1&subpartNode=21:501149>
25. Learned LA, Reynolds MG, Wassa DW, Li Y, Olson VA, Karem K, et al. Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. *Am J Trop Med Hyg* 2005;73(2):428–34. [PubMed: 16103616]

Figure 1.

Diagram of the Study Design for Vaccinia-Naïve (Panel A) and Vaccinia-Immune (Panel B) Volunteers. Dryvax® challenge occurred 12 weeks following primary immunization in each group. For some analyses (*), the 9 individuals who received one dose of Dryvax® at Day 0 were added to the Placebo and Dryvax® group.

Figure 2.

Lesion Progression of Skin Reactions (Panel A) and Take Category Following Dryvax[®] Challenge in Vaccinia-Naïve (Panel B) and Vaccinia-Immune (Panel C) Volunteers. Take category 0 = no take skin reaction; take category 1 = modified take skin reaction without vesicle; take category 2 = modified take skin reaction with vesicle; take category 3 = primary take skin reaction. One group (*) includes 11 volunteers in the placebo group after Dryvax[®] challenge and 9 volunteers in 2-dose Dryvax[®] group after their first Dryvax[®].

Figure 3. MVA Impact on Vaccinia Replication. Semi-quantitative viral shedding following Dryvax[®] challenge in vaccinia-naïve (Panel A) and vaccinia-immune (Panel B) subjects. The values are the mean titer of the log₁₀ pfu/ml and the bars represent the 95% confidence interval.

Figure 4. MVA Immunogenicity. Neutralizing antibody data at 50% geometric mean titer for vaccinia-naïve and vaccinia-immune subjects (Panel A) and the mean percent of total CD4+ T cells (Panel B) or total CD8+ T cells (Panel C) that produced interferon- γ and IL-2 in response to stimulation with vaccinia or MVA. The bars represent the 95% confidence interval.

Figure 5.

Individual immune parameters in vaccinia-naïve subjects following Dryvax® challenge. The percent of total CD8+ T cells that produced interferon- γ and IL-2 by vaccine group in response to stimulation with vaccinia or MVA (Panel A). The response to the extracellular enveloped virus (EEV) proteins B5R (Panel B) and A33R (Panel C) by vaccine group. The box plots show the median, 10th, 25th, 75th, and 90th percentiles.

Table 1**Contraindications to Dryvax® that excluded volunteers from study participation**

Diseases or conditions that cause immunodeficiency or in close physical contact (household or work) with such an individual
History of eczema or in close physical contact with such an individual
Acute or chronic skin conditions such as atopic dermatitis, burns, impetigo, or varicella zoster
Pregnant or breast-feeding women
Household member who is pregnant
Close contact with <1 year old
Serious, life-threatening allergies to antibiotics polymixin B, streptomycin, tetracycline, neomycin, or cidofovir
Allergy to eggs or blood products or hypersensitivity to probenidic or sulfa-containing medications

Table 2

Self-assessed local and systemic reactogenicity are represented. The number and percent of volunteers with symptoms rated as mild, moderate, or severe are included following primary injection series of either MVA or placebo (Panel A) and following Dryvax[®] (Panel B)

A. REACTOGENICITY FOLLOWING PLACEBO OR MVA INJECTION SERIES				
	Vaccinia-naïve		Vaccinia-immune	
	Placebo N=11	All MVA N=54	Placebo N=30	All MVA N=45
T>100	0 (0.0%)	2 (3.7%)	0 (0.0%)	0 (0.0%)
Headache	4 (36.4%)	17(31.5%)	8 (26.7%)	7 (15.6%)
Malaise	6 (54.5%)	22(40.7%)	5 (16.7%)	13(28.9%)
Myalgia	3 (27.3%)	16(29.6%)	5 (16.6%)	10(22.2%)
Chills	3 (27.3%)	3 (5.6%)	1 (3.3%)	1 (2.2%)
Nausea	4 (36.4%)	7 (13.0%)	4 (13.3%)	1 (2.2%)
Pain at Site	2 (18.2%)	26(48.1%)	5 (16.7%)	19(42.2%)*
Underarm Pain	1 (9.1%)	6 (11.1%)	0 (0.0%)	5 (11.1%)
Underarm Swelling	2 (18.2%)	1 (1.9%)	0 (0.0%)	2 (4.4%)
Pruritus[†]	1 (9.1%)	3 (5.6%)	1 (3.3%)	6 (13.3%)
B. REACTOGENICITY FOLLOWING DRYVAX[®] CHALLENGE				
	Vaccinia-naïve		Vaccinia-immune	
	Dryvax[®] Only[‡] N=20	All MVA/Dryvax[®] N=43	Dryvax[®] Only N=28	All MVA/Dryvax[®] N=39
T>100	3 (15.0%)	0 (0.0%)*	2 (7.1%)	0 (0.0%)
Headache	7 (35.0%)	18 (41.9%)	11 (39.3%)	8 (20.5%)
Malaise	15 (75.0%)	23 (53.5%)	11 (39.3%)	8 (20.5%)
Myalgia	10 (50.0%)	19 (44.2%)	6 (21.4%)	8 (20.5%)
Chills	4 (20.0%)	1 (2.3%)*	3 (10.7%)	2 (5.1%)
Nausea	9 (45.0%)	10 (23.3%)	1 (3.6%)	1 (2.6%)
Pain at Site	16 (80.0%)	22 (51.2%)*	5 (17.9%)	15 (38.5%)
Underarm Pain	16 (80.0%)	20 (46.5%)*	5 (17.9%)	4 (10.3%)
Underarm Swelling	12 (60.0%)	13 (30.2%)*	2 (7.1%)	1 (2.6%)
Pruritus[†]	18 (90.0%)	39 (90.7%)	25 (89.3%)	26 (66.7%)*
Mean diameter of peak erythema(cm)	3.665	1.860*	2.386	1.223*
Mean diameter of peak induration(cm)	2.565	1.818*	1.643	1.192*
Mean diameter of peak lesion size(cm)	1.145	0.820*	0.821	0.692

* p-value <0.05 comparing all MVA to placebo, 2-sided Fisher's exact test

[†] Indicates local reactogenicity

[‡] Includes 11 volunteers in the placebo group after Dryvax[®] challenge and 9 volunteers in 2 dose Dryvax[®] group after their first Dryvax[®] vaccination.

* p-value <0.05 comparing all MVA/Dryvax[®] to Dryvax[®] only, 2-sided Fisher's exact test

[†] Indicates local reactogenicity