Article

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**Abstract**

The replication-competent recombinant vesicular stomatitis virus (rVSV)–based vaccine expressing a Zaire ebolavirus (ZEBOV) glycoprotein was selected for rapid safety and immunogenicity testing before its use in West Africa.

ORIGINAL ARTICLE

# Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe — Preliminary Report

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## ABSTRACT

### BACKGROUND

The replication-competent recombinant vesicular stomatitis virus (rVSV)–based vaccine expressing a *Zaire ebolavirus* (ZEBOV) glycoprotein was selected for rapid safety and immunogenicity testing before its use in West Africa.

### METHODS

We performed three open-label, dose-escalation phase 1 trials and one randomized, double-blind, controlled phase 1 trial to assess safety, side-effect profile, and immunogenicity of rVSV-ZEBOV at various doses in 158 healthy adults in Europe and Africa. All participants were injected with doses of vaccine ranging from 300,000 to 50 million plaque-forming units (PFU) or placebo.

### RESULTS

No serious vaccine-related adverse events were reported. Mild-to-moderate early-onset reactogenicity was frequent but transient (median, 1 day). Fever was observed in up to 35% of vaccinees. Vaccine viremia was detected within 3 days in 103 of 110 participants (94%) receiving 3 million PFU or more; rVSV was not detected in saliva or urine. In the second week after injection, arthritis affecting one to four joints developed in 11 of 51 participants (22%) in Geneva, with pain lasting a median of 8 days; 2 self-limited cases occurred in 40 participants (5%) in Hamburg, Germany, and Kilifi, Kenya. The virus was identified in one synovial-fluid aspirate and in skin vesicles of 2 other vaccinees, showing peripheral viral replication in the second week after immunization. ZEBOV-glycoprotein–specific antibody responses were detected in all the participants, with similar glycoprotein-binding antibody titers but significantly higher neutralizing antibody titers at higher doses.

### CONCLUSIONS

In these studies, rVSV-ZEBOV was reactogenic but immunogenic after a single dose and warrants further evaluation for safety and efficacy. (Funded by the Wellcome Trust and others; ClinicalTrials.gov numbers, NCT02283099, NCT02287480, and NCT02296983; Pan African Clinical Trials Registry number, PACTR201411000919191.)

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*The contributions of the authors, committee members, and other members of the VSV Ebola Consortium (VEBCON) are described in the Supplementary Appendix, available at NEJM.org.

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In August 2014, after the current outbreak of Ebola virus disease was declared a public health emergency of international concern by the World Health Organization (WHO), the Canadian government donated 800 vials of the replication-competent recombinant vesicular stomatitis virus (rVSV)–vectored *Zaire ebolavirus* (rVSV-ZEBOV) candidate vaccine to the WHO. The VSV Ebola Consortium (VEBCON) was created under the auspices of the WHO to initiate phase 1 studies to facilitate rapid progression to phase 2 and 3 trials in affected countries.[1]

Live replicating viral vaccines elicit humoral and cellular immune responses against viral pathogens.[2,3] A single injection of 10 million plaque-forming units (PFU) of rVSV-ZEBOV protected nonhuman primates exposed to lethal doses of *Zaire ebolavirus*.[4-7] Vesicular stomatitis virus belongs to the Rhabdoviridae family.[8] In livestock, wild-type VSV causes vesicles and ulcerations of the oral tissues, feet, and teats.[9] Human infections are rare and asymptomatic or typically cause mild influenza-like illness, although more severe infections have been described.[9-14] The wild-type virus is not endemic in Africa and Europe.[15,16] The preclinical safety record of the rVSV vector is encouraging: among roughly 80 immunized nonhuman primates, none had detectable toxic effects.[3] Viremia associated with rVSV-ZEBOV was detected on day 2 only, suggesting rapid viral clearance through the innate immune response. Safety in immunocompromised hosts was assessed in a few nonhuman primates infected with the human immunodeficiency virus[6] and in mice with severe combined immunodeficiency.[17] None of the animals had detectable illness after immunization. Viral shedding in saliva and urine was not observed.[3]

To assess the safety and immunogenicity of various doses of rVSV-ZEBOV in countries with or without previous outbreaks of Ebola virus disease, we initiated parallel, harmonized VEBCON trials in Lambaréné, Gabon; Kilifi, Kenya; Hamburg, Germany; and Geneva. We report the initial safety and immunogenicity data emerging from these ongoing studies.

## METHODS

### STUDY DESIGNS AND PARTICIPANTS

The studies in Lambaréné, Kilifi, and Hamburg were open-label, uncontrolled, phase 1 trials designed to assess the safety, side-effect profiles, and immunogenicity of ascending doses of rVSV-ZEBOV vaccine (BPSC1001) at doses ranging from 300,000 to 20 million PFU in healthy adults of both sexes between the ages of 18 and 55 years. The Geneva study was a double-blind, randomized, placebo-controlled, phase 1 trial assessing the safety and immunogenicity of the rVSV–ZEBOV vaccine at doses of 10 million and 50 million PFU in healthy adults between the ages of 18 and 65 years. Full details regarding the study centers, entry criteria, and procedures are provided in the individual study protocols, available with the full text of this article at NEJM.org. The studies were reviewed and approved by the respective national competent authorities, local ethics committees, the German authority for genetic engineering, and the WHO research ethics review committee. All the participants provided written informed consent. An independent consortium-wide data and safety monitoring board provided oversight.

All four studies were investigator-initiated trials sponsored by each local institution. The Wellcome Trust provided funding through a grant to the WHO. Other individual funding sources are outlined in the Supplementary Appendix, available at NEJM.org. A total of 800 vaccine doses were donated to the WHO by the Public Health Agency of Canada. Funding bodies and the vaccine manufacturers were not involved in the analysis of the data, nor did they contribute to the preparation or writing of the manuscript.

### VACCINE AND PLACEBO

The rVSV-ZEBOV vaccine was developed by the Canadian National Microbiology Laboratory and was licensed to BioProtection Systems (a subsidiary of NewLink Genetics). The vaccine was subsequently sublicensed to Merck, which has assumed responsibility for ongoing research and development. The vaccine was manufactured at IDT Biologika in Dessau-Rosslau, Germany, and stored in a manner consistent with good manufacturing practices. The same lot (no. 003 05 13), which was dispensed in single-dose vials as 100 million PFU per milliliter, was sent from Canada to Geneva and subsequently to the other sites. (Additional details regarding reconstitution are provided in the Supplementary Appendix.) Placebo syringes containing 0.5 ml of saline were packaged identically.

### VACCINATION

Injections were administered intramuscularly into the deltoid. Dose-escalation studies were staggered (for details, see the Methods section in the Supplementary Appendix). In the Lambaréné cohort, participants received doses of 300,000 PFU or 3 million PFU. In Hamburg, participants received doses of 3 million PFU or 20 million PFU. In Kilifi, participants received a dose of 3 million PFU. In Geneva, the first 19 run-in participants received a single open-label injection of 10 million PFU and were observed for at least 1 week. Thereafter, participants who were planning to deploy to Ebola-affected regions were randomly assigned in a 1:1 ratio in a double-blind fashion to receive a vaccine dose of either 10 million PFU or 50 million PFU, whereas those who were not planning to deploy to such regions were randomly assigned in a 1:1:1 ratio to receive either vaccine dose or placebo. (An overview of the four trials is provided in Fig. S4 in the Supplementary Appendix.)

### SAFETY MONITORING

Injection-site and systemic reactogenicity and medication use were recorded for 7 days after injection and at follow-up (days 14 and 28). Clinical and laboratory evaluations were performed during each study visit (for details, see the Methods section in the Supplementary Appendix). Laboratory analyses included a complete blood count and measurements of creatinine, C-reactive protein, and liver function. Adverse events were listed for each participant according to the Common Terminology Criteria for Adverse Events and the *Medical Dictionary for Regulatory Activities* and are reported individually and in aggregate.

### INVESTIGATION OF ARTHRITIS AND SKIN LESIONS

After the observation of arthralgia in some Geneva participants, all but the first participant with swollen joints or axial involvement in Geneva underwent joint imaging (by means of ultrasonography or magnetic resonance imaging [MRI]), and all but two participants were referred to a rheumatologist. Arthritis was confirmed if the study team observed swelling or imaging revealed effusion. Participants in Geneva who had skin lesions underwent biopsy, swabbing, or puncture of lesions.

### DETECTION OF rVSV

We developed quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays (TaqMan) targeting the VSV nucleoprotein gene (see the Methods section in the Supplementary Appendix). Results are expressed in copies per milliliter. In the dose-escalations trials, rVSV viral loads were monitored from day 0 to day 28, including daily sampling of plasma, urine, and saliva through day 7 in Hamburg. In Lambaréné and Kilifi, total RNA from plasma, urine (400 $\mu$l), and saliva (200 $\mu$l) (Viral Transport Kit, BD) was stored in TRIzol LS Reagent (Life Technologies) and analyzed at St. George's University of London. In Hamburg, samples were assessed on site. In Geneva, RT-PCR was performed on days 1, 3, and 7 on all plasma specimens, on saliva and urine in the first 20 participants vaccinated with 10 million PFU and 10 participants vaccinated with 50 million PFU, and later on skin vesicles and synovial fluid. RT-PCR assay to detect rVSV was performed on oral lesions observed in Hamburg and Geneva. Virus isolation was performed in Geneva by means of plaque assay on Vero E6 cells from selected samples and confirmed on PCR and immunostaining (see the Methods section in the Supplementary Appendix).

### IMMUNOGENICITY

We assessed serum samples on days 0 and 28 after injection. We performed the enzyme-linked immunosorbent assay (ELISA) for ZEBOV-glycoprotein–specific antibodies using the homologous Zaire-Kikwit strain glycoprotein (following the standard operating procedure of the U.S. Army Medical Research Institute of Infectious Diseases [SOP AP-03-35-00]) or inactivated whole virions of the Zaire-Guéckédou strain. The relative amounts of ZEBOV-glycoprotein–specific antibodies were reported as end-point titers or geometric mean concentration of arbitrary ELISA units per milliliter. Neutralizing antibodies were detected with the use of VSV pseudovirions expressing the luciferase reporter gene complemented by glycoprotein from the ZEBOV 95 Kikwit strain, as described previously,[18] or infectious ZEBOV isolate Mayinga. (For detailed descriptions, see the Methods section in the Supplementary Appendix.)

### STATISTICAL ANALYSIS

We determined the frequencies of all adverse events according to study center and dose group.

Categorical variables are described with counts and percentages, and continuous variables with means and standard deviations or medians and interquartile ranges for skewed variables. Antibody responses are reported as the geometric mean titer or geometric mean concentration with 95% confidence intervals. We obtained reverse cumulative distributions by plotting for each possible value of the titer the proportion of participants with a titer greater than this value. We used Fisher's exact test, the Mann–Whitney test, and the Kruskal–Wallis test or Spearman's correlation coefficient to calculate intergroup associations. For immunogenicity, we compared geometric mean titers or concentrations, seropositivity rates, and seroresponse rates between days 0 and 28 using Wilcoxon's test for paired data and McNemar's test. We used Spearman's correlation coefficient to assess the correlation between assays. Although at the time of this analysis clinical investigators and participants in the randomized, controlled trial were unaware of study-group assignments, the blind was broken after the 3-month visit for the 11 participants with arthritis. The statistical analysis plans are provided in the study protocols at NEJM.org.

## RESULTS

### STUDY POPULATIONS

A total of 158 participants received either vaccine (150 participants) or placebo (8 participants) in the three dose-escalation studies from November 17, 2014, through January 19, 2015, and in the Geneva randomized, controlled trial from November 10, 2014, through December 9, 2014, before a safety-driven study hold and subsequent resumption of vaccination with a lower dose (3 million PFU) (Fig. S4 in the Supplementary Appendix). The study populations are described in Table 1, and in Table S2 in the Supplementary Appendix. In Geneva, the run-in participants and those who underwent randomization were compared for baseline characteristics and adverse-event outcomes. In the absence of significant differences, pooled results are reported. Vaccine was administered in doses as follows: 300,000 PFU in 20 participants, 3 million PFU in 49 participants, 10 million PFU in 35 participants, 20 million PFU in 30 participants, and 50 million PFU in 16 participants. A total of 138 participants had been followed for at least 4 weeks at the time of this analysis (Fig. S4 in the Supplementary Appendix). The results reported here are from interim databases for ongoing trials.

### SAFETY

#### *Serious Adverse Events*

There were no serious adverse events associated with the vaccine. Three hospitalizations due to malaria occurred in Lambaréné.

#### *Acute Reactogenicity*

Solicited and unsolicited adverse events were frequent (Table 2). Of the 138 participants who were followed for at least 4 weeks, 124 (90%) had at least one adverse event, with the majority of events reported as mild or moderate. Grade 3 symptoms were reported in 3 of 20 participants (15%) in Kilifi, 2 of 20 (10%) in Hamburg, and 12 of 51 (24%) in Geneva; none were reported in Lambaréné. Local reactogenicity was common but generally mild. Most adverse events appeared early (median, 1 day; interquartile range, <24 hours to 1 day), subsided rapidly (median, 1 day; interquartile range, <24 hours to 1 day), and were alleviated with the use of acetaminophen or nonsteroidal antiinflammatory drugs as needed. The incidence and intensity of the events varied according to both the dose and the study site: objective fever was reported in 5 of 20 participants (25%) in Hamburg, 13 of 51 (25%) in Geneva, 6 of 20 (30%) in Kilifi, and 2 of 39 (5%) in Lambaréné. At a given dose, such as 3 million PFU, inflammatory reactions were more frequently reported in hospitalized participants in Hamburg than in Lambaréné (Table 2).

#### *Biologic Monitoring*

Hematologic changes were observed in all participants who were monitored during the first days after vaccination (Table S3 in the Supplementary Appendix). In Lambaréné, transient leukocytopenia was observed in 12 of 20 participants (60%) receiving 300,000 PFU and 8 of 19 participants (42%) receiving 3 million PFU; lymphocytopenia was observed in 2 of 19 participants (11%) in the group receiving 3 million PFU.

In Hamburg, an asymptomatic drop in the number of circulating lymphocytes was observed 1 day after vaccination in all participants and was resolved by day 2 ($P<0.01$ for the compari-

son between screening vs. day 1). The decrease was unrelated to dose, reactogenicity, or substantial viremia, indicating biologic activity even at a dose of 3 million PFU. Among the 51 participants in Geneva, 36 (71%) had a decreased number of circulating lymphocytes on day 1, and 27 (53%) had a decreased number of neutrophils on day 3, with rapid resolution of both conditions (Table S3 in the Supplementary Appendix). Monocytosis on day 3 and a transient reduction in platelets were also observed. Liver-function and creatinine levels remained unchanged.

*Viremia and Viral Shedding*

Low levels of rVSV-ZEBOV RNA were identified in plasma on RT-PCR assay on days 1 to 3 in most participants who were tested (Fig. 1, and Table S4 in the Supplementary Appendix). Participants with positive results for rVSV on PCR assay ranged from 8 of 20 (40%) among Lambaréné vaccinees immunized with 300,000 PFU to 42 of 51 (82%) among Geneva vaccinees. In Lambaréné, 18 of 19 participants (95%) who received a dose of 3 million PFU had detectable viremia on day 1 or 2; 15 of 19 participants (79%) had complete resolution by day 7. The same pattern was observed in Kilifi, where 18 of 20 participants (90%) had values that were below the detection level by day 7. In Hamburg, all participants who received doses of 3 million PFU and 20 million PFU who were monitored daily until day 7 had detectable viremia, with peaks on day 2 or 3 and complete resolution by day 5.

All but eight plasma specimens from all studies were negative by day 7, and infectious virus was not recovered from any blood specimen tested. In the Geneva study, there was no correlation between peak viremia and vaccine dose, age, sex, frequency or intensity of adverse events, or lymphocytopenia. Viral RNA was not detected in saliva or urine samples at any site; occasional oral lesions (Table 2) were all negative for rVSV on PCR assay.

*Arthritis*

In Geneva, 11 of 51 participants (22%) with no previous history of joint disease had an onset of arthralgia a median of 11 days (interquartile range, 9 to 13) after injection; 8 participants had

**Table 1. Characteristics of the Patients at Baseline.***

| Characteristic | Hamburg | | Lambaréné | | Kilifi | Geneva | | | All Participants (N=138) |
|---|---|---|---|---|---|---|---|---|---|
| | Vaccine, 3 Million PFU (N=10) | Vaccine, 20 Million PFU (N=10) | Vaccine, 300,000 PFU (N=20) | Vaccine, 3 Million PFU (N=19) | Vaccine, 3 Million PFU (N=20) | Vaccine, 10 Million PFU (N=35) | Vaccine, 50 Million PFU (N=16) | Placebo (N=8) | |
| Sex — no. (%) | | | | | | | | | |
| Male | 6 (60) | 7 (70) | 14 (70) | 17 (89) | 14 (70) | 21 (60) | 11 (69) | 4 (50) | 94 (68) |
| Female | 4 (40) | 3 (30) | 6 (30) | 2 (11) | 6 (30) | 14 (40) | 5 (31) | 4 (50) | 44 (32) |
| Age — yr | | | | | | | | | |
| Mean | 32±8 | 40±9 | 30±8 | 27±6 | 34±7 | 42±11 | 43±14 | 39±12 | 36±10 |
| Range | 24–47 | 24–51 | 24–47 | 20–44 | 27–49 | 21–59 | 24–59 | 23–58 | 21–59 |
| Race — no. (%)† | | | | | | | | | |
| Asian | 1 (10) | 0 | 0 | 0 | 0 | 2 (6) | 1 (6) | 0 | 4 (3) |
| Black | 0 | 0 | 20 (100) | 19 (100) | 17 (85) | 2 (6) | 0 | 0 | 58 (42) |
| White | 9 (90) | 10 (100) | 0 | 0 | 3 (15) | 29 (83) | 15 (94) | 8 (100) | 74 (54) |
| Body-mass index‡ | 25±3 | 24±3 | 23±3 | 22±3 | 24±4 | 25±4 | 25±3 | 25±4 | 24±3 |

* Plus–minus values are means ±SD. Listed are values for the 138 participants who were followed for at least 4 weeks. There were no significant differences among the groups except for race between all dose groups in Lambaréné and Kilifi and those in Hamburg and Geneva ($P<0.001$ for all comparisons). PFU denotes plaque-forming units.

† Race was self-reported. Two participants in the Geneva cohort receiving 10 million PFU were Hispanic.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

received 10 million PFU and 3 had received 50 million PFU. These participants presented with asymmetric involvement of a median of 2 (range, 1 to 4) peripheral joints, with swelling on physical examination and as seen on ultrasonography as tenosynovitis or bursitis in at least 1 joint (Table S5 in the Supplementary Appendix). Three participants had axial involvement; of these, 1 had evidence of arthritis on MRI. Thus, arthritis was confirmed in 9 of 11 participants. Pain was

**Table 2. Adverse Events within 14 Days after Receipt of rVSV-ZEBOV Vaccine or Placebo.***

| Adverse Event | Hamburg | | Lambaréné | | Kilifi | Geneva | | |
|---|---|---|---|---|---|---|---|---|
| | Vaccine, 3 Million PFU (N=10) | Vaccine, 20 Million PFU (N=10) | Vaccine, 300,000 PFU (N=20) | Vaccine, 3 Million PFU (N=19) | Vaccine, 3 Million PFU (N=20) | Vaccine, 10 Million PFU (N=35) | Vaccine, 50 Million PFU (N=16) | Placebo (N=8) |
| | *number of participants (percent)* | | | | | | | |
| **Any event** | | | | | | | | |
| None | 0 | 1 (10) | 5 (25) | 2 (11) | 3 (15) | 1 (3) | 0 | 2 (25) |
| Mild | 5 (50) | 5 (50) | 12 (60) | 9 (47) | 8 (40) | 11 (31) | 3 (19) | 5 (62) |
| Moderate | 4 (40) | 3 (30) | 3 (15) | 8 (42) | 6 (30) | 14 (40) | 10 (62) | 1 (12) |
| Severe | 1 (10) | 1 (10) | 0 | 0 | 3 (15) | 9 (26) | 3 (19) | 0 |
| **Solicited injection-site reaction** | | | | | | | | |
| Erythema | | | | | | | | |
| None | 9 (90) | 8 (80) | 20 (100) | 19 (100) | 20 (100) | 35 (100) | 15 (94) | 8 (100) |
| Mild | 1 (10) | 2 (20) | 0 | 0 | 0 | 0 | 1 (6) | 0 |
| Swelling or induration | | | | | | | | |
| None | 9 (90) | 9 (90) | 20 (100) | 19 (100) | 19 (95) | 34 (97) | 16 (100) | 8 (100) |
| Mild | 1 (10) | 1 (10) | 0 | 0 | 1 (5) | 1 (3) | 0 | 0 |
| Pain | | | | | | | | |
| None | 4 (40) | 5 (50) | 18 (90) | 11 (58) | 10 (50) | 9 (26) | 3 (19) | 8 (100) |
| Mild | 6 (60) | 5 (50) | 2 (10) | 8 (42) | 8 (40) | 26 (74) | 12 (75) | 0 |
| Moderate | 0 | 0 | 0 | 0 | 2 (10) | 0 | 1 (6) | 0 |
| **Solicited systemic symptom** | | | | | | | | |
| Objective fever | | | | | | | | |
| None | 8 (80) | 7 (70) | 20 (100) | 17 (89) | 14 (70) | 26 (74) | 12 (75) | 8 (100) |
| Mild | 2 (20) | 3 (30) | 0 | 1 (5) | 6 (30) | 9 (26) | 4 (25) | 0 |
| Moderate | 0 | 0 | 0 | 1 (5) | 0 | 0 | 0 | 0 |
| Subjective fever | | | | | | | | |
| None | 9 (90) | 10 (100) | 19 (95) | 12 (63) | 17 (85) | 13 (37) | 6 (38) | 7 (88) |
| Mild | 1 (10) | 0 | 1 (5) | 4 (21) | 3 (15) | 14 (40) | 5 (31) | 1 (12) |
| Moderate | 0 | 0 | 0 | 3 (16) | 0 | 6 (17) | 4 (25) | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 2 (6) | 1 (6) | 0 |
| Chills | | | | | | | | |
| None | 7 (70) | 7 (70) | 20 (100) | 17 (89) | 17 (85) | 18 (51) | 6 (38) | 8 (100) |
| Mild | 3 (30) | 3 (30) | 0 | 2 (11) | 1 (5) | 7 (20) | 4 (25) | 0 |
| Moderate | 0 | 0 | 0 | 0 | 1 (5) | 7 (20) | 5 (31) | 0 |
| Severe | 0 | 0 | 0 | 0 | 1 (5) | 3 (9) | 1 (6) | 0 |

**Table 2. (Continued.)**

| Adverse Event | Hamburg | | Lambaréné | | Kilifi | Geneva | | |
|---|---|---|---|---|---|---|---|---|
| | Vaccine, 3 Million PFU (N=10) | Vaccine, 20 Million PFU (N=10) | Vaccine, 300,000 PFU (N=20) | Vaccine, 3 Million PFU (N=19) | Vaccine, 3 Million PFU (N=20) | Vaccine, 10 Million PFU (N=35) | Vaccine, 50 Million PFU (N=16) | Placebo (N=8) |
| | *number of participants (percent)* | | | | | | | |
| Myalgia | | | | | | | | |
| None | 2 (20) | 4 (40) | 20 (100) | 13 (68) | 16 (80) | 12 (34) | 5 (31) | 6 (75) |
| Mild | 5 (50) | 6 (60) | 0 | 3 (16) | 3 (15) | 15 (43) | 6 (38) | 1 (12) |
| Moderate | 3 (30) | 0 | 0 | 3 (16) | 1 (5) | 5 (14) | 5 (31) | 1 (12) |
| Severe | 0 | 0 | 0 | 0 | 0 | 3 (9) | 0 | 0 |
| Headache | | | | | | | | |
| None | 3 (30) | 5 (50) | 16 (80) | 10 (53) | 11 (55) | 14 (40) | 8 (50) | 5 (62) |
| Mild | 6 (60) | 4 (40) | 3 (15) | 6 (32) | 6 (30) | 11 (31) | 4 (25) | 3 (38) |
| Moderate | 1 (10) | 1 (10) | 1 (5) | 3 (16) | 2 (10) | 10 (29) | 3 (19) | 0 |
| Severe | 0 | 0 | 0 | 0 | 1 (5) | 0 | 1 (6) | 0 |
| Fatigue | | | | | | | | |
| None | 6 (60) | 9 (90) | 10 (50) | 10 (53) | 19 (95) | 13 (37) | 8 (50) | 6 (75) |
| Mild | 3 (30) | 1 (10) | 7 (35) | 5 (26) | 1 (5) | 5 (14) | 5 (31) | 2 (25) |
| Moderate | 0 | 0 | 3 (15) | 4 (21) | 0 | 16 (46) | 3 (19) | 0 |
| Severe | 1 (10) | 0 | 0 | 0 | 0 | 1 (3) | 0 | 0 |
| Gastrointestinal symptom | | | | | | | | |
| None | 10 (100) | 8 (80) | 15 (75) | 15 (79) | 17 (85) | 26 (74) | 12 (75) | 8 (100) |
| Mild | 0 | 1 (10) | 5 (25) | 3 (16) | 3 (15) | 5 (14) | 4 (25) | 0 |
| Moderate | 0 | 1 (10) | 0 | 1 (5) | 0 | 3 (9) | 0 | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 1 (3) | 0 | 0 |
| **Unsolicited adverse event** | | | | | | | | |
| Oral vesicles | | | | | | | | |
| None | 8 (80) | 9 (90) | 20 (100) | 19 (100) | 20 (100) | 35 (100) | 16 (100) | 8 (100) |
| Mild | 2 (20) | 1 (10) | 0 | 0 | 0 | 0 | 0 | 0 |
| Arthralgia† | | | | | | | | |
| None | 10 (100) | 9 (90) | 20 (100) | 12 (63) | 16 (80) | 30 (86) | 14 (88) | 8 (100) |
| Mild | 0 | 0 | 0 | 4 (21) | 0 | 3 (9) | 2 (12) | 0 |
| Moderate | 0 | 1 (10) | 0 | 3 (16) | 4 (20) | 2 (6) | 0 | 0 |
| Arthritis‡ | | | | | | | | |
| None | 9 (90) | 10 (100) | 20 (100) | 19 (100) | 19 (95) | 27 (77) | 13 (81) | 8 (100) |
| Mild | 0 | 0 | 0 | 0 | 0 | 4 (11) | 0 | 0 |
| Moderate | 1 (10) | 0 | 0 | 0 | 1 (5) | 0 | 1 (6) | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 4 (11) | 2 (12) | 0 |

* Listed are values for the 138 participants who were followed for at least 4 weeks. Percentages may not total 100 because of rounding.
† Arthralgia was observed during the first week after immunization.
‡ Arthritis was observed during the second week after immunization.

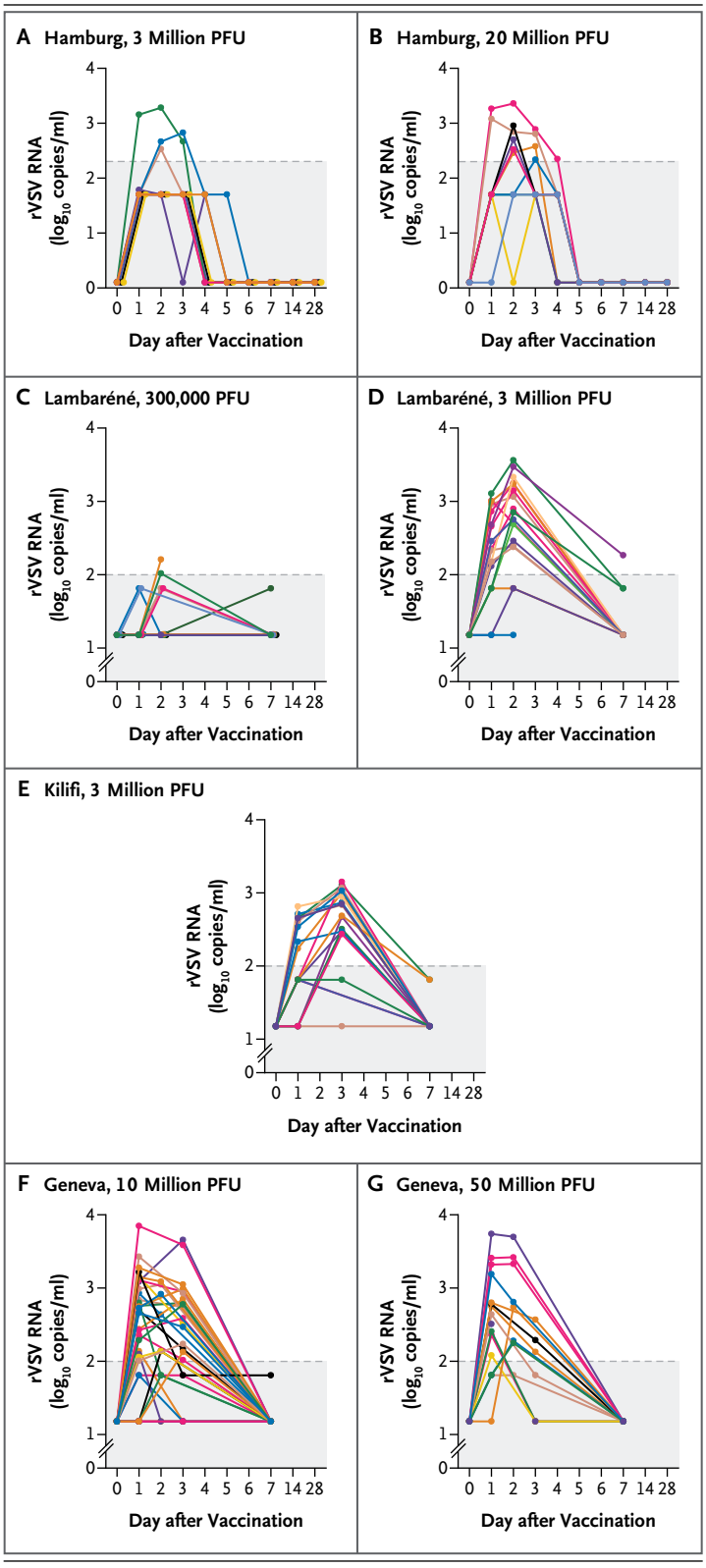



**Figure 1. Plasma Recombinant Vesicular Stomatitis Virus (rVSV) Viremia in the Four Study Cohorts.**

The presence of rVSV viremia was assessed with the use of quantitative reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays (TaqMan) of total RNA derived from plasma. The viral load is expressed as $\log_{10}$ rVSV RNA copies per milliliter. Each measurement included no-template and standard controls. The shaded area indicates values that are below the limit of quantification (≤100 copies per milliliter of RNA in Lambaréné, Kilifi, and Geneva and ≤200 copies per milliliter in Hamburg). Panels A and B show the plasma viral load through day 28 for the two Hamburg cohorts that received doses of 3 million and 20 million plaque-forming units (PFU), with daily sampling from day 1 to day 7. Panels C and D show individual viremia patterns in two cohorts that received doses of 300,000 and 3 million PFU, as monitored in Lambaréné. Plasma was analyzed between day 0 and day 2 and on day 7 in all participants. Panel E shows the plasma viral load on days 0, 1, 3, and 7 among participants in Kilifi who received a vaccine dose of 3 million PFU. Panels F and G show rVSV RNA copy numbers on days 0, 1, 3, and 7 in participants in Geneva who received vaccine doses of 10 million PFU or 50 million PFU.

often migratory and generally mild, with a median duration of 8 days (interquartile range, 6 to 13) but lasting more than 3 months in 1 participant. The functional effect of the arthritis was moderate, with a median score of 2.5 (interquartile range, 1.8 to 3.3) on the Routine Assessment of Patient Index Data (RAPID3),[19] on a scale ranging from 1.0 to 10.0, with higher values indicating a greater severity. Results also indicated low inflammatory disease activity, with a median score of 1.8 (interquartile range, 1.7 to 2.0) on the Disease Activity Score in 44 joints (DAS44),[20] on a scale ranging from 0 to 10, with higher values indicating more active disease. Post-vaccination elevations in autoantibodies were not observed. A knee arthrocentesis in 1 patient yielded 40 ml of fluid with 7190 leukocytes (80% monocytes) and rVSV at 1200 copies per milliliter on PCR assay, whereas synovial viral and bacterial cultures and rVSV viremia remained negative. No association between the presence of arthritis and vaccine dose, age, sex, earlier arthralgia, or peak viremia was observed among the Geneva participants. Two self-limited cases of arthritis were observed, with one each in Hamburg and Kilifi. (Details regarding these cases are provided in the Supplementary Appendix.)

*Skin Lesions*

Among the 11 participants in Geneva with arthritis, a mild maculopapular rash mainly on the limbs developed in 3 participants between days 7 and 9 and lasted 7 to 15 days (Fig. 2A, subpanel a). The rash was associated with a few tender vesicles on fingers or toes (Fig. 2B, subpanel d). Histologic analysis of one papule revealed a dermal T-lymphocytic infiltrate (Fig. 2A, subpanels b and c). Vesicular lesions reflected subepidermal dermatitis with necrotic keratinocytes (Fig. 2B, subpanel d) containing abundant VSV antigens (Fig. 2B, subpanel f).[21] Among the 3 participants with rash, rVSV was identified on RT-PCR in all 3 up to day 17, and infectious rVSV was isolated from a specimen with the highest RNA level 9 days after immunization (Fig. 2C). Concomitantly obtained plasma samples remained negative, showing local replication. At other VEBCON trial sites, investigators screened participants for rVSV-associated dermatologic findings, but none were observed.

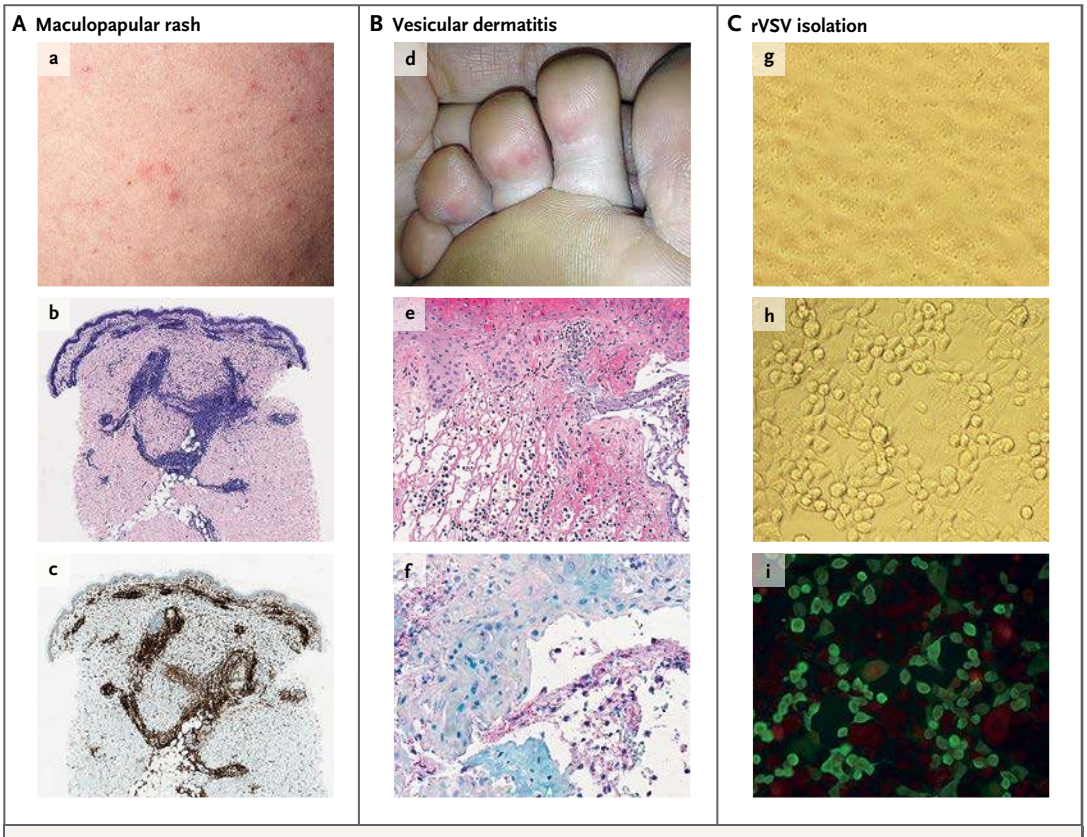



**Figure 2. Vaccine-Induced Maculopapular and Vesicular Dermatitis.**

Panel A shows maculopapular lesions on the thigh of a participant (subpanel a). Histologic analysis of one papule revealed a dermal T-lymphocytic infiltrate (subpanel b, hematoxylin and eosin staining) characterized mainly by CD3+ T cells (subpanel c). Panel B shows vesicular lesions on the plantar side of the toes of a participant (subpanel d). Histologic analysis shows subepidermal vesicular dermatitis with vacuolar degeneration, keratinocyte necrosis, acute and lymphohistiocytic inflammation and fibrin (subpanel e, hematoxylin and eosin staining). Higher-power magnification of the same area shows abundant immunostaining of rVSV antigens associated with cellular debris and inflammatory infiltrate (subpanel f, immunoalkaline phosphatase technique[21] using a mouse antibody against VSV and Naphthol fast red substrate). Panel C shows the isolation of rVSV-ZEBOV, with a cytopathic effect induced on Vero E6 cells after culture with control medium (subpanel g) or a swabbed skin vesicle (subpanels h and i) observed by means of either light microscopy (subpanels g and h) or after immunostaining (subpanel i) for VSV matrix protein (green, rVSV-ZEBOV–infected cells, with Evans blue counterstaining; red, noninfected cells).

### ZEBOV-GLYCOPROTEIN–SPECIFIC ANTIBODY RESPONSES

Serum antibodies induced by rVSV-ZEBOV were assessed with the use of four distinct assays. Baseline antibody levels were generally low, with outliers. Low-level baseline seropositivity was identified in 12 of 23 participants (52%) in Lambaréné (Fig. 3A, and Tables S6 through S9 in the Supplementary Appendix).

Four weeks after immunization, ZEBOV-glycoprotein–specific antibodies were detected on ELISA in all vaccinees with similar anti-glycoprotein geometric mean titers (Fig. 3A, and Table S6 in the Supplementary Appendix) and distribution, as seen on reverse cumulative distribution curves (Fig. 3E). The lowest dose (300,000 PFU) was immunogenic in Lambaréné, with a higher proportion of participants with a low response than among those receiving higher doses (Fig. 3A through 3D). Rates of seropositivity (0 to 53%) and titers were lower with the use of whole-virion ELISA (Fig. 3B, and Table S7 and Fig. S5 in the Supplementary Appendix), which identified baseline antibodies reacting with nucleocapsid or matrix proteins, but not glycoprotein, in samples from Lambaréné (Fig. S6 in the Supplementary Appendix).

On pseudovirion neutralization assay assessing the 50% serum neutralization capacity (PsVNA50), neutralizing antibodies were absent at baseline (including among participants in Lambaréné) but were elicited in 76 of 91 vaccinees (84%) (Fig. 3C, and Table S8 in the Supplementary Appendix). With the use of infectious ZEBOV particles, low-level neutralizing antibodies (≥1:8) were detected at baseline in 6 of 20 participants (30%) in Hamburg, 3 of 20 (15%) in Kilifi, and 10 of 38 (26%) in Lambaréné (Fig. 3D, and Table S9 in the Supplementary Appendix). The two assays showed significant increases in neutralizing antibodies after any dose of rVSV-ZEBOV (Tables S8 and S9 in the Supplementary Appendix).

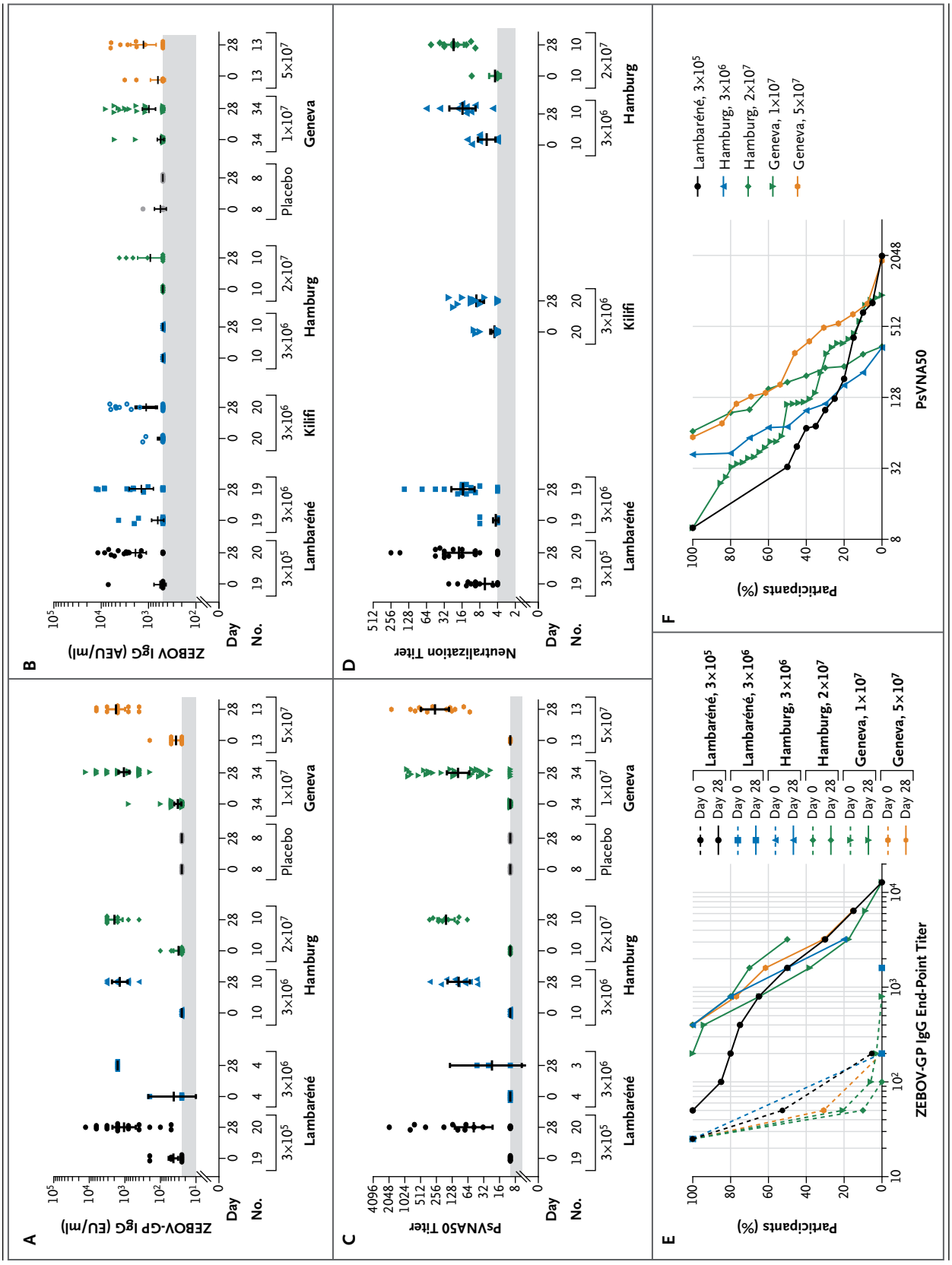
Despite strong correlations between antibody titers on glycoprotein ELISA and on PsVNA50 (Fig. S7 in the Supplementary Appendix), significant differences were observed. In the Geneva study, a dose of 50 million PFU elicited similar titers of glycoprotein-binding antibodies as did 10 million PFU, with geometric mean titers of 1780 (95% confidence interval [CI], 1048 to 3022) and 1064 (95% CI, 757 to 1495), respectively, but significantly higher PsVNA50 antibody titers, with geometric mean titers of 273 (95% CI, 157 to 475) and 99 (95% CI, 62 to 159) ($P=0.02$). The influence of increasing doses on the distribution of neutralizing antibodies was confirmed on reverse cumulative distribution (Fig. 3F) and correlation analyses (Tables S10 and S11 and Fig. S8 in the Supplementary Appendix). Thus, higher doses of rVSV-ZEBOV elicited similar glycoprotein-binding titers but higher neutralizing-antibody titers.

**Figure 3 (facing page). Glycoprotein Antibody Titers, According to Vaccine Dose, Study Site, and Assay.**

Individual antibody titers were assessed at baseline and 28 days after vaccination in 138 participants, according to study site and dose group. The number of samples tested and the vaccine dose (in PFUs) are indicated for each site below the graphs in Panels A through D; the shaded areas indicate negative titers. Antibodies were measured on enzyme-linked immunosorbent assay (ELISA) against the homologous glycoprotein (GP) of the Zaire-Kikwit strain (ZEBOV, Panel A) or inactivated whole virions of the Zaire-Guéckédou strain (Panel B). Results are expressed as end-point titers (Panel A) or the geometric mean concentration of arbitrary ELISA units (AEU) per milliliter (Panel B). Neutralizing antibodies were detected with the use of rVSV pseudovirion neutralization assay assessing the 50% serum neutralization capacity (PsVNA50) complemented by homologous glycoprotein (Panel C) or with infectious ZEBOV isolate Mayinga (Panel D). Geometric mean titers and 95% confidence intervals are shown for each study site, dose group, and time point. The results of glycoprotein ELISA (Panel E) and pseudoneutralization (Panel F) were expressed as the reciprocal of the highest dilution showing a positive result. The curves represent the distribution of individual antibody titers in each cohort. The dashed lines indicated baseline titers in Panel E.

## DISCUSSION

The Zaire-variant candidate vaccine rVSV-ZEBOV expresses a viral surface glycoprotein that has immunogenic potential after a single dose for populations at risk for Ebola virus disease, as well as possibly for postexposure prophylaxis.[4,7,17,22] It is a replication-competent chimeric virus for which human-safety data were limited to a single postexposure case report[21] at the time of the initiation of this study. We describe the safety and immunogenicity results for rVSV-ZEBOV in African and European populations, collected in four harmonized, ongoing trials, including three



dose-escalating trials and one randomized, controlled trial.

Acute inflammatory symptoms were expected after immunization with rVSV-ZEBOV. The frequency and intensity of such symptoms were study-dependent. At a vaccine dose of 3 million PFU, objective fever was reported in 20 to 30% of vaccinees in Hamburg and Kilifi but in only 11% in Lambaréné. An influence of dose on adverse events was noted between a dose of 300,000 PFU and 3 million PFU in Lambaréné but not between higher doses at other sites. It is likely that the early onset, short duration, and responsiveness to symptomatic treatment of these symptoms will facilitate acceptance of the vaccine, but vaccine-induced fever should be anticipated if rVSV-ZEBOV is administered to contacts of patients infected with Ebola virus disease.

Levels of rVSV RNA were transiently detected in early blood samples, suggesting that innate responses, especially those involving the type I interferon pathway, help to limit viral replication.[23-26] Viral seeding of joints and skin, mostly identified in the Geneva cohort, was unexpected. It showed that viral dissemination and replication can occur and persist for up to 2 to 3 weeks after immunization — in other words, that early innate responses may not always be sufficient for complete viral control. Replication appeared to be restricted to permissive tissues: viral RNA remained below detection in plasma and peripheral-blood mononuclear cells, and replicating virus was retrieved only from skin vesicles. Interestingly, skin vesicles in livestock infected with VSV or foot-and-mouth virus occur at similar locations, reflecting the relative resistance of keratinocytes to type I interferon.[27] The pattern of rVSV-ZEBOV replication in humans thus may be defined by the permissiveness of rVSV replication.[28,29]

In the Geneva cohort, arthritis was confirmed in 9 of 51 participants (18%) and suspected in another 2. Two cases were reported among 40 participants in Hamburg and Kilifi (5%), albeit at a lower intensity and of shorter duration than in Geneva. Possible mechanisms of virus-induced arthritis include autoimmunity, lytic effects of infected synovial cells, and the deposition of immune complexes. The induction of autoimmunity is not supported by the rapid onset (<2 weeks) and the lack of vaccine-induced pathogenic antibodies. We cannot rule out immune-complex deposition, but the detection of rVSV RNA in synovial fluid showed the seeding of rVSV-ZEBOV into joints. Since arthralgia or arthritis is not elicited by VSV and was not reported with other rVSV constructs that have been tested to date, the pathophysiology of the chimeric rVSV-ZEBOV vaccine may include features attributable to both its VSV and ZEBOV glycoprotein components. Although pain may be prolonged and relapse may occur, the prognosis of viral vaccine-induced arthritides is considered favorable on the basis of experience with rubella vaccination.[30] Accordingly, the VEBCON data and safety monitoring board concluded on January 1, 2015, that the trials could proceed as originally planned (including doses up to 100 million PFU) once informed-consent forms were updated.

The rVSV-ZEBOV vaccine generated glycoprotein-binding antibodies in all participants at any dose, showing its immunogenicity in humans. Doses containing as few as 300,000 PFU may be sufficient to elicit glycoprotein-binding antibodies. Preexisting antibodies to ZEBOV nucleocapsid or matrix proteins (Fig. S6 in the Supplementary Appendix) conferred no advantage for the induction of glycoprotein-specific responses. Neutralizing antibodies were also generated in most participants, and a dose–response effect was shown. Despite similar glycoprotein-binding antibody titers, higher vaccine doses elicited higher titers of neutralizing antibodies. Since the relative roles of neutralizing and glycoprotein-binding antibodies in protection against Ebola virus disease are unknown, we cannot conclude whether higher vaccine doses are required for optimal protection. A comparison of anti–glycoprotein antibody titers detected in this study (in the Hamburg cohort receiving 20 million PFU) with those reported with a chimpanzee adenovirus vector[31] revealed similar values. This finding suggests that the two vaccines that are currently undergoing testing in West Africa may induce humoral responses of the same order of magnitude.

These investigations have contributed to the dose-selection process performed by the vaccine manufacturers and have raised the awareness of investigators, members of institutional review boards, and regulators about the specific adverse events to be expected with the use of vaccines. They have also resulted in the introduction of

safety-driven changes in the protocols for the ongoing phase 2 and 3 studies.

In conclusion, first results of rapidly implemented, parallel phase 1 studies including in-depth safety investigations showed that the rVSV–ZEBOV vaccine is reactogenic but immunogenic at doses ranging from 300,000 to 50 million PFU in African and European volunteers, with higher titers of neutralizing antibodies at higher doses. The viral dissemination in skin and joints that was observed in some participants needs to be balanced with the possible benefit offered by this vaccine in the potential control of outbreaks of Ebola virus disease.

The views expressed in this article are those of the authors and do not necessarily represent the position or policies of the WHO, the U.S. Army, the Centers for Disease Control and Prevention, or the Kenya Medical Research Institute.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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## APPENDIX

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