Tularemia vaccine: Safety, reactogenicity, "Take" skin reactions, and antibody responses following vaccination with a new lot of the Francisella tularensis live vaccine strain - A phase 2 randomized clinical Trial

Mark Mulligan, Emory University
Jack T. Stapleton, University of Iowa
Wendy A. Keitel, Baylor College of Medicine
Sharon E. Frey, St. Louis University
Wilbur H. Chen, University of Maryland
Nadine Rouphael, Emory University
Srilatha Edupuganti, Emory University
Allison Beck, Emory University
Patricia L Winokur, University of Iowa
Hana M. El Sahly, Baylor College of Medicine

Only first 10 authors above; see publication for full author list.

Journal Title: Vaccine
Volume: Volume 35, Number 36
Publisher: Elsevier: 12 months | 2017-08-24, Pages 4730-4737
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.vaccine.2017.07.024
Permanent URL: https://pid.emory.edu/ark:/25593/td7jk

Final published version: http://dx.doi.org/10.1016/j.vaccine.2017.07.024

Copyright information:
© 2017 The Authors
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Accessed November 19, 2019 9:00 PM EST
Tularemia Vaccine: Safety, Reactogenicity, “Take” Skin Reactions, and Antibody Responses following Vaccination with a New Lot of the *Francisella tularensis* Live Vaccine Strain - A Phase 2 Randomized Clinical Trial

Mark J. Mulligan, MD\(^a\),\(^\otimes\), Jack T Stapleton, MD\(^b\), Wendy A. Keitel, MD\(^c\), Sharon E. Frey, MD\(^d\), Wilbur H. Chen, MD\(^e\), Nadine Rouphael, MD\(^a\), Srilatha Edupuganti, MD\(^a\), Allison Beck, PA-C\(^a\), Patricia L Winokur, MD\(^b\), Hana M. El Sahly, MD\(^c\), Shital M. Patel, MD\(^c\), Robert L. Atmar, MD\(^c\), Irene Graham, MD\(^d\), Edwin Anderson, MD\(^d\), Samer S. El-Kamary, MBBS\(^e\), Marcela F. Pasetti, PhD\(^e\), Marcelo B. Sztein, MD\(^e\), Heather Hill\(^f\), Johannes B. Goll\(^f\), and the DMID 08-0006 Tularemia Vaccine Study Group\(^g\)

\(^a\)The Hope Clinic of the Emory Vaccine Center, Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, 500 Irvin Court, Suite 200, Decatur, GA 30030, USA

\(^b\)University of Iowa, SW54-4, GH, 200 Hawks Drive, Iowa City, IA 52242, USA

\(^c\)Departments of Molecular Virology and Microbiology, and Medicine, Baylor College of Medicine, Houston, TX, BCM MS 280, One Baylor Plaza, Houston, TX 77030, USA

\(^d\)Division of Infectious Diseases, Allergy and Immunology, Saint Louis University School of Medicine, 1100 South Grand Boulevard, DRC-8, St. Louis, MO 63104, USA

\(^e\)Center for Vaccine Development, University of Maryland School of Medicine, 685 W. Baltimore St., Suite 480, Baltimore, MD 21201, USA

\(^f\)The Emmes Corporation, 401 North Washington Street, Suite 700, Rockville, MD 20850, USA

Abstract

Background—Tularemia is caused by *Francisella tularensis*, a gram-negative bacterium that has been weaponized as an aerosol. For protection of personnel conducting biodefense research, the United States Army required clinical evaluation of a new lot of tularemia live vaccine strain manufactured in accordance with Current Good Manufacturing Practices.

\(^\otimes\)Corresponding author: Mark J. Mulligan, MD, FIDSA Distinguished Professor of Medicine, The Hope Clinic of the Emory Vaccine Center, Emory University, 500 Irvin Court, Suite 200, Decatur, GA 30030, Office phone: 404-712-9046, Office fax: 404-499-9728, mark.mulligan@emory.edu.

\(^\#\)Members of the DMID 08-0006 Tularemia Vaccine Study Group who are co-authors (in addition to the byline authors listed above) include: Jennifer A. Whitaker, MD, \(^1\); and Idefonso Tellez, MD (deceased)\(^1\).

ClinicalTrials.gov identifier NCT01150695

The authors had no conflicts of interest to report.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
**Methods**—A phase 2 randomized clinical trial compared the new lot (DVC-LVS) to the existing vaccine that has been in use for decades (USAMRIID-LVS). The vaccines were delivered by scarification to 228 participants. Safety, reactogenicity, take and/or antibody levels were assessed on days 0, 1, 2, 8, 14, 28, 56, and 180.

**Principal Results**—Both vaccines were safe and had acceptable reactogenicity profiles during six months of follow-up. There were no serious or grade 3 and 4 laboratory adverse events. Moderate systemic reactogenicity (mostly headache or feeling tired) was reported by ~23% of participants receiving either vaccine. Injection site reactogenicity was mostly mild itchiness and pain. The frequencies of vaccine take skin reactions were 73% (95% CI, 64, 81) for DVC-LVS and 80% (95% CI, 71, 87) for USAMRIID-LVS. The 90% CI for the difference in proportions was −6.9 % (−16.4, 2.6). The rates of seroconversion measured by microagglutination assay on days 28 or 56 were 94% (95% CI, 88, 98; n = 98/104) for DVC-LVS and 94% (95% CI, 87, 97; n = 103/110) for USAMRIID-LVS (p=1.00). Day 14 sera revealed more rapid seroconversion for DVC-LVS relative to USAMRIID-LVS: 82% (95% CI, 73, 89) versus 55% (95% CI, 45, 65), respectively (p<0.0001).

**Major conclusions**—The DVC-LVS vaccine had similar safety, reactogenicity, take and antibody responses compared to the older USAMRIID vaccine, and was superior for early (day 14) antibody production. Vaccination take was not a sensitive surrogate for seroconversion in a multi-center study where personnel at five research clinics performed assessments.

**Keywords**
Tularemia; LVS; bacterial; take; vaccine; *Francisella tularensis*

“I know of no other infection of animals communicable to man that can be acquired from sources so numerous and so diverse. In short, one can but feel that the status of tularemia, both as a disease in nature and of man, is one of potentiality.”—R. R. Parker, Proceedings of the Fifth Pacific Congress, 1934.

**Introduction**

*Francisella tularensis* is transmitted to humans via insect vectors such as ticks, mosquitoes, or biting deer flies, and by handling contaminated animal products or carcasses, particularly rabbits and rodents. 2–4 Fewer than 200 cases per year occur in the United States, mostly during summer and in the central South. *F. tularensis* is a non-motile, facultative intracellular, Gram-negative bacillus that is among the most infectious pathogenic bacteria known, causing human disease after inoculation, inhalation, or ingestion. As few as 10 inhaled organisms cause disease.5,6 Two biovars have been described: type A, *F. tularensis* biovar tularensis, is the most common isolate in North America and is highly virulent in humans with an overall mortality rate of 5–7% if untreated;7 type B, *F. tularensis* biovar holarctica, is more common outside of the United States and is relatively avirulent.8 Type A *F. tularensis* is a dangerous potential biological weapon due to its high infectivity, ease of dissemination, and significant capacity to cause illness and death1,9,10 and has been classified as a category A bioterrorism agent by the Centers for Disease Control and
Prevention. Japan, the Soviet Union, the United States and others have weaponized the bacterium as an aerosol.\(^1\)

There is no effective licensed vaccine available for prevention of tularemia although \(>5,000\) people have received an investigational live, attenuated vaccine that prevents typhoidal disease (fever, headache, malaise, prostration, and often cough and chest pain) and ameliorates ulceroglandular disease (local ulceration with regional adenopathy, fever, chills, and malaise) in laboratory workers.\(^11\) Experimentally-infected subjects who were treated with streptomycin when symptoms developed could clearly be re-infected upon repeat experimental challenge.\(^5\) Vaccination with killed vaccine did not prevent local lesions following cutaneous experimental challenge but did reduce systemic manifestations of infection\(^4\) and provided no protection following respiratory challenge.\(^6\) Live attenuated vaccines were first used in humans in the Soviet Union in 1942 and were brought to the United States by Shope in 1956.\(^12\) The live vaccine strain (LVS) delivered by scarification provided significant protection against typhoidal illness following respiratory challenge.\(^6\) Following vaccination, the appearance of an erythematous papule, vesicle or eschar has been correlated with immunity, and this skin finding is designated a take reaction. In a retrospective analysis of all tularemia cases at the Fort Detrick research facility, it was concluded that typhoidal tularemia virtually disappeared following LVS administration (falling from 5.7 to 0.27 cases/1,000 employee years at risk) and ulceroglandular disease decreased significantly in clinical severity.\(^13\)

The United States Army Medical Research Institute of Infectious Diseases-LVS (USAMRIID-LVS) vaccine\(^12\) has been used under an investigational new drug (IND) application for decades\(^5,6\) but the supply is limited and aging. Therefore the Department of Defense contracted with Dynport Vaccine Company (DVC) to produce a new lot of LVS using Current Good Manufacturing Practices (cGMP). After pre-clinical work\(^14\), a phase 1 trial of escalating doses of the new DVC-LVS lot administered to 70 subjects concluded that vaccine delivery by scarification was safe, tolerable, and produced superior antibody responses than subcutaneous delivery.\(^15\) The goals of the phase 2 trial reported here were to directly compare the new DVC-LVS lot to USAMRIID-LVS in 228 subjects by defining the kinetics of antibody responses; comparing injection site reactions following scarification with vaccines versus saline; and correlating antibody responses with take.

### Methods

#### Study design

We conducted a phase 2, multi-center, double-blind, randomized trial comparing the \(F.\) \textit{tularensis} DVC-LVS and USAMRIID-LVS vaccines. The planned study population was approximately 220 healthy male and non-pregnant female subjects aged 18–45 years. Eligibility criteria are at ClinicalTrials.gov - identifier NCT01150695. The protocol and consent form were reviewed by the US Food and Drug Administration, and approved and monitored by the sites’ institutional review boards. Subjects were randomized to receive a single dose of DVC-LVS or USAMRIID-LVS by scarification in one arm. All received normal saline (NS) by scarification in the contralateral arm (a control). Following vaccination (day 0) subjects were followed for safety, reactogenicity, immunological
responses and/or take on days 1, 2, 8, 14, 28, 56, and 180. The primary objectives were: to assess the frequency of serious adverse events (SAEs) and Grade 3 and 4 laboratory values following vaccinations; to assess the frequency of take (defined below) following vaccinations; and to assess the rate of seroconversion following vaccinations as measured by a tularemia-specific microagglutination assay.

**Sponsor and Study sites**

The study was sponsored by the US government’s National Institutes of Health/National Institute of Allergy and Infectious Diseases/Division of Microbiology and Infectious Diseases (NIH/NIAID/DMID), conducted in collaboration with USAMRIID, and performed at five DMID Vaccine and Treatment Evaluation Units (VTEUs): Emory University School of Medicine; University of Iowa; Baylor College of Medicine; Saint Louis University; and University of Maryland School of Medicine. A central laboratory (University of Maryland School of Medicine) performed the serum antibody assays and a central statistical and data-coordinating center (EMMES Corporation) performed the data management.

**Vaccines**

USAMRIID-LVS was produced in the 1960s by the National Drug Biologic Research Company (Swiftwater, Pennsylvania). The lyophilized vaccine (lot number NDBR 101 lot 4) contained live, attenuated *F. tularensis*; modified casein partial hydrolysate medium; sucrose gelatin agar stabilizer solution; and glucose cysteine hemin agar. Prior to reconstitution, all USAMRIID-LVS vials were tested for container integrity using the “SPARK” test in which a high frequency generator is used to test for the presence of a vacuum. Vials not passing this test were discarded. Two mL of sterile water for injection (WFI) were used to reconstitute the vaccine. Each multi-dose vial of USAMRIID-LVS contained ~$1.0 \times 10^9$ CFU/mL.

DVC-LVS was produced using cGMP by DVC (Frederick, Maryland). NDBR 101 Lot 4 of USAMRIID-LVS was the seed material. DVC-LVS Lot No. 703-0505-020 was used in this study. Lyophilized DVC-LVS was reconstituted with 0.25 mL WFI. The reconstituted vaccine contained ~$1 \times 10^9$ CFU/mL in 10% sucrose, 1.9% gelatin and 10 mM potassium phosphate. After reconstitution, vaccines were stored at 2–8°C and used within 8 hours. NS for the control scarifications was supplied as single dose vials (Hospira Inc., Lake Forest, Illinois).

After cleansing the skin with acetone, the vaccines or NS were administered by scarification of the volar surface of the forearm midway between the wrist and the elbow. 100 μl of solution (containing $10^8$ CFU or saline) was drawn into a tuberculin syringe; the needle was removed and one drop was placed on the skin. A bifurcated needle (Fisher BioServices, Washington, DC) was used to puncture the skin 15 times through the drop in an 0.5cm² area. A trace amount of blood at the site was evidence of successful vaccine delivery. The site was allowed to air dry for 3–5 minutes before blotting with a dry sterile gauze.
Safety and reactogenicity monitoring

Unsolicited adverse events (AE) and abnormal laboratory values were assessed through day 28 and SAEs were collected through day 180. Solicited systemic and injection site reactogenicity symptoms were recorded by the subjects on diary cards through day 28 and followed until resolution. Study staff performed examinations at clinic visits and recorded dimensions of injection site erythema and induration.

Antibody assay

Antibody responses were measured by a tularemia-specific microagglutination assay performed as previously described\textsuperscript{14,15} on days 0, 14, 28, 56 and 180.

Take reactions

Take was defined as the development of an erythematous papule, vesicle, and/or eschar with or without underlying induration by days 7–9 as assessed by the VTEUs’ staffs. As a secondary assessment of take, digital photographs of both scarification sites (vaccine and control) were obtained at clinic visits, uploaded to a central study database, and assessed by an independent take evaluation committee of three blinded experts familiar with reactions after scarification. Both the site staff and the take evaluation committee categorized vaccination site reactions at each visit into one of several categories (Supplemental Table 1).

Statistical analyses

For the safety and reactogenicity analyses, all vaccinated subjects were included. The intention-to-treat (ITT) analyses of immunogenicity and take included all subjects who had at least one available measurement. The per protocol (PP) analyses of immunogenicity and take included subjects who met all inclusion and exclusion criteria, whose day 28 and 56 visits occurred within protocol-specified windows (between days 26–30 and days 49–63, respectively), and who contributed both pre- and post-vaccination blood samples for which valid results were reported. For safety and seroconversion categorical variables, 95% exact (Clopper-Pearson) confidence intervals (CI) were calculated for each proportion and comparisons were carried out using a two-sided Fisher’s exact test. The geometric mean and associated 95% CI was used to summarize microagglutination titers. The primary immunogenicity endpoint was the proportion of subjects who seroconverted (>4-fold rise in titer) at days 28 or 56. Peak microagglutination titer was defined as the maximum titer reported for days 14, 28 or 56. Treatment comparisons were carried out using a two-sided t-test. For the take endpoint a 90% CI of the difference in positive take proportions (Wald asymptotic CI) was determined and used to evaluate significance. Except for take rate comparisons, an individual alpha level of 5% was applied for all tests. Except as noted once below, results across the five sites did not differ and aggregate results are presented.

Results

Enrollment and Demographics

Enrollment occurred between September 2010 and May 2011, and the last study visit was in January 2012. The dispositions for the 509 participants who were enrolled and screened, and
the 228 (45%) eligible participants who were randomized and vaccinated, are summarized (Figure 1). 113 participants received DVC-LVS and 115 received USAMRIID-LVS. Unless noted, this report presents the PP analyses as the ITT results were similar. The randomized subjects’ demographic characteristics were well balanced between the two study groups (Supplemental Table 2).

Safety

Both vaccines appeared safe. There were no SAEs and no grade 3 or 4 laboratory AEs. No subject in either group had changes in hematology or chemistry values greater than mild in severity. 152 unsolicited AEs were reported by 107 subjects: 53 of 113 subjects (47%) in the DVC-LVS group and 54 of 115 (47%) in the USAMRIID-LVS group (p=1.00) (Supplemental Figure 1A). One migraine headache after DVS-LVS vaccination was severe but unrelated; the remaining 151 unsolicited AEs were mild to moderate. The unsolicited AEs were graded as related (n=44 events) or unrelated (n=108) to vaccination. 16 of 113 (14%) DVC-LVS subjects had vaccine-related AEs while 26 of 115 (23%) USAMRIID-LVS subjects had related AEs (p=0.124) (Supplemental Figure 1B); most common were vaccination site satellite lesions that were mild in severity and resolved without sequelae (n=7 and 13, respectively).

Reactogenicity

Injection site reactogenicity was mostly mild and was similar for the two vaccines (Table 1A). The most prevalent injection site reactions for both vaccines were itchiness and pain. Pain occurred in 49.6% of vaccine sites and 11.5% of NS control sites in the DVC-LVS group, as compared to 44.3% of vaccine sites and 12.2% of NS sites in the USAMRIID-LVS group (p values 0.507 and 1.00, respectively). Severe vaccine site reactions based on lesion diameter (erythema or induration >5cm) occurred for 5 (4.4%) DVC-LVS scarification sites and 4 (3.5%) USAMRIID-LVS sites (p=0.747) (Table 1B); there were no severe reactions for NS. In general for both vaccines the vaccination site lesions had largely healed by 21–28 days. No lymphangitis was observed.

There were no significant differences in none/mild versus moderate/severe solicited systemic symptoms (p=0.118) (Table 2). Symptoms were mostly mild and occurred over the first few days post-vaccination. In the DVC-LVS group, 2 subjects (2%) experienced severe and 27 subjects (24%) experienced moderate systemic reactions. In the USAMRIID-LVS group, 3 subjects (3%) experienced severe and 25 subjects (22%) experienced moderate systemic reactions. Headache and feeling tired were the two most commonly reported systemic reactogenicity symptoms, each occurring in approximately one-third of subjects.

Take

LVS delivered by scarification was expected to cause small injection site take reactions. Since take has been used as a surrogate for immunogenicity, we evaluated the utility of take in this multi-center clinical trial. Before study start investigators and staff received fairly extensive training in assessing scarification site lesions including written definitions and sample photographs for lesion categories including erythema, papule, vesicle, pustule, and eschar (Supplemental Table 1). Based on the blinded investigator categorizations of the
scarification site appearances at each study visit (representative digital photographs, Figure 2), the data center determined take rates for the two vaccines and NS. USAMRIID-LVS resulted in higher take rates than DVC-LVS in both ITT and PP analyses (~80% compared to ~70%) (Table 3A) but the null hypothesis of equality of positive take proportions between DVC-LVS and USAMRIID-LVS was rejected with alpha=0.10 for only ITT but not PP. For the PP population, the 90% CI for the difference in proportions was −6.9 % (−16.4, 2.6%). Take rates at the 5 clinical trial sites differed significantly after controlling for vaccine type (p=0.022). While two of the clinics had take rates between 88–93%, the other three clinics’ take rates ranged between 66–73%. The five clinics’ categorizations of the NS control sites were not different (p=0.184), nor were there differences in their microagglutination seroconversion rates (p=0.35, PP population).

For a secondary objective, an independent take committee also categorized lesion appearances by reviewing digital photographs of the vaccination sites. While DVC-LVS resulted in higher take rates than USAMRIID-LVS (~93% compared to ~87%) in both ITT and PP analyses (Table 3B), the null hypothesis of equality of positive take proportions between DVC-LVS and USAMRIID-LVS was rejected with alpha=0.10 for only PP but not ITT. For the PP population, the 90% CI for the difference in proportions was 7.1% (0.6, 13.7%). Interestingly, assessments of the contralateral arm NS control scarification sites by both clinic investigators (physical exam) and the take committee (photographs) met the take definition for 10% of subjects.

**Antibody response**

The two vaccines demonstrated no difference in the primary immunogenicity endpoint (proportion seroconverting at day 28 or 56): 94% for both vaccines. Interestingly, at an earlier time-point (day 14), DVC-LVS had a higher seroconversion proportion than DVC-USAMRIID: 85 /104 subjects (82% [95% CI, 73, 89]) versus 60/109 subjects (55% [95% CI, 45, 65]), respectively (p<0.0001) (Figure 3A).

The peak (day 14, 28, or 56) geometric mean titers (GMT) did not differ: 1166 (95% CI: 911, 1493) for DVC-LVS, and 1225 (95% CI: 946, 1586) for USAMRIID-LVS (p=0.786). Similar to the seroconversion rate, the day 14 GMT for DVC-LVS was significantly higher than for DVC-USAMRIID: 144 (95% CI, 118, 176) vs. 70 (95% CI, 59, 82), respectively (p<0.0001) (Figure 3B). For all other time-points there were no significant seroconversion or GMT differences (Supplemental Table 3). There was no interaction between either vaccine and gender or vaccine and age for microagglutination antibody log fold rises from baseline across post-vaccination days when fitting a linear mixed-effects model (not shown).

We assessed the overlaps between take and seroconversion for 214 subjects across both vaccine groups who had measurements by both methods (investigator lesion assessment and antibody seroconversion). Among these subjects, 201 seroconverted; 13 did not. When seroconversion is taken as the gold standard, the true positive rate (sensitivity) for takes was 77% (154 of 201 seroconverters had takes) and the true negative rate (specificity) was 23% (only 3 of 13 non-seroconverters were scored as not having takes) (Supplemental Table 4).
Discussion

The new DVC-LVS vaccine lot performed well relative to the USAMRIID-LVS vaccine. Both vaccines appeared safe and were tolerated well in the study population. The generally accepted serological correlate of effective immunization (seroconversion in the microagglutination assay) was observed in 94% of subjects with both vaccines.

At the earliest post-vaccination time-point (day 14), antibody seroconversion and GMT were significantly better for the new lot of vaccine. The more rapid development of an antibody response could be an advantage for either post-exposure prophylaxis or in an ongoing *F. tularensis* bioterrorism event or outbreak scenario where rapid pre-exposure prophylaxis is desired. The reason for the more rapid development of antibody response with DVC-LVS is unknown. One hypothesis for the day 14 effect is that the decades-old USAMRIID-LVS vaccine has begun to lose replication capacity and takes longer to “get out of the gate” – although both vaccines continued to pass ongoing stability testing programs. Alternatively, since the new DVC-LVS vaccine was derived from USAMRIID-LVS and was produced using modern cGMP methods and in a fermenter rather than shaker flasks, lineage and/or processing differences could be responsible for the different behavior of the vaccines.

In this multicenter trial, the correlation between antibody seroconversion and vaccine take was poor. If seroconversion is used as the gold standard, take underestimated vaccine response and resulted in false negatives. Furthermore, specificity was low (23%) and 10% of NS injection reactions were categorized as takes. In addition, there were site-to-site differences in the observed take rates. These take results occurred despite utilizing experienced vaccine investigators, including several with experience in successfully assessing takes following scarification with the smallpox vaccine Dryvax®, and an extensive education/training program including photographic examples to guide lesion assessments in the clinics. It is difficult to determine what additional steps could be taken to improve take sensitivity and specificity under similar circumstances in the future. Possibly if investigators without a long history of assessing LVS takes are required to administer the vaccine, more emphasis will need to be placed on the immunogenicity results than the take rate.

One limitation of this report is its immunological focus on the antibody response on day 14 and later. However additional analyses of the innate and cellular responses are being conducted. These studies may provide additional mechanistic insights into the more rapid DVC-LVS antibody response.

In conclusion, the safety, tolerability and antibody responses exhibited by the two vaccines were very similar suggesting that the DVC-LVS vaccine could replace the current USAMRIID-LVS vaccine if the latter vaccine were to become unavailable for human use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
Acknowledgments

The National Institutes of Health/National Institute of Allergy and Infectious Diseases/Division of Microbiology and Infectious Diseases (NIH/NIAID/DMID) provided financial support to the Vaccine and Treatment Evaluation Units [VTEUs] for the conduct of the research and preparation of the article. The sponsor had a role in study design; in interpretation of data; and in the writing of the report. The sponsor did not have a role in the collection or analysis of the data, or the decision to submit the article for publication.

The investigators wish to thank: the volunteer study participants at the five VTEU sites; the US DOD including Dr. Judith Pace-Templeton and Dr. Ellen Boudreau for sharing expertise and providing the two study vaccines; and the following faculty and staff who are acknowledged for their contributions to the study: JoAnn Sadowski, William Emery, Beverly Weaver, Alexis Daughtery, Becky Gerkin, Vickie Grimes, Pamela Lankford-Turner, Mary Bower (Emory University); Geraldine Dull, Michelle Rodenberg, Nancy Wagner, Dan Zhao, and other members of the Iowa VTEU and Clinical Research Unit staff (University of Iowa); Tracey Lanford, Jan Brown, Veeral Kumar, Innocent Mbawuike, Yolanda Rayford, Diane Nino, Herbert DuPont, and the staff of the Baylor College of Medicine Vaccine Research Center (Baylor College of Medicine); Thomas Pacatte, Sabrina DiPiazza, Linda Eggemeyer, Kathleen Goldmacher, Sharon Irby-Moore, Jan Tennant, Carol Duane, Karla Mosby, Irene Graham and Edwin Anderson (Saint Louis University); Sandra Getlein, Lisa Chrisley (University of Maryland); and Robert Johnson, PhD, Marianne Baker, RN, MSN (NIH/NIAID/DMID).

The three expert members of the independent take committee are deserving of special thanks for the generous donation of their time and expertise to this clinical trial: Kathryn Edwards, MD, Sarah H. Sell and Cornelius Vanderbilt Chair in Pediatrics, Vanderbilt Vaccine Research Program, Vanderbilt University; Gregory Deye, MD, NIH/NIAID/DMID; and Mark J. Goldberg, MD, CIV, US, USA MEDCOM USAMRIID. The authors also thank Dr. Judith Pace-Templeton and Dr. Ellen Boudreau from the US Department of Defense.

Funding sources The study was funded by the US government’s NIH/NIAID/DMID, and conducted at five DMID VTEUs: Emory University in Atlanta, Georgia, contracts HHSN272200800005C and HHSN272201300018I; University of Iowa in Iowa City, contracts HHSN272200800008C and HHSN272201300020I; University of Maryland in Baltimore, Maryland, contracts HHSN272200800001C and HHSN272201300022I; St. Louis University, contracts HHSN272200800003C and HHSN272201300021I; and Baylor College of Medicine in Houston, Texas, contracts HHSN272200800002C and HHSN272201300015I. The immunogenicity assays were performed by a central research laboratory at the University of Maryland. The statistical and data coordinating center was the EMMES Corporation, contracts HHSN272200800013C and HHSN2722012000031. Additional support was provided by: the Georgia Research Alliance (MJM); the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Numbers UL1TR000454 (Emory) and U54TR001356 (Iowa); and NCRR Grant K12-RR-023250 (WHC).

Literature Cited


Figure 1. CONSORT diagram
The diagram details the dispositions for all participants who were enrolled and screened, and for eligible participants who were randomized and vaccinated.

1 All enrolled and vaccinated subjects are included in the safety data analyses.
2 The intention to treat (ITT) analysis population for the immunogenicity analyses includes all subjects.
3 The per protocol (PP) analysis population for the immunogenicity analyses include subjects who met all inclusion and exclusion criteria, whose visits 7 and 8 were within protocol-specified windows (between days 26–30 and 49–63, respectively) and who contributed both pre- and post-vaccination blood samples for testing for which valid results were reported. Fourteen of the 228 subjects randomized and vaccinated were excluded from the per protocol immunogenicity analyses.
Figure 2. Digital Photographs of Representative Scarification Sites Categorized as Takes, by Study Visit

For selected days post-scarification, vaccine scarification sites and one saline scarification site are shown. The appearances of scarification sites for the two study vaccines were similar and so vaccine groups are not specified here. Take was defined as the development of an erythematous papule, vesicle, and/or eschar with or without underlying induration by days 7–9 as assessed by the VTEUs’ staffs.
Figure 3. Antibody Responses in the Microagglutination Antibody Assay. A
Proportions of subjects seroconverting (achieving a 4-fold or greater rise from day 0 baseline) by study group and study day. B. Geometric mean titers by study group and study day.
Table 1

A. Functional Grading
Mild events required minimal or no treatment and did not interfere with the subject’s daily activities. Moderate events resulted in a low level of inconvenience or concern and caused some interference with functioning. Severe events interrupted a subject’s usual daily activity and may have required systemic drug therapy or other treatment. Severe events are usually incapacitating.

<table>
<thead>
<tr>
<th>Reactogenicity Parameter</th>
<th>DVC-LVS</th>
<th>USAMRIID-LVS</th>
<th>DVC-LVS Control</th>
<th>USAMRIID-LVS Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at Site</td>
<td>N</td>
<td>None n (%)</td>
<td>Mild n (%)</td>
<td>Moderate n (%)</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>57 (50.4)</td>
<td>50 (44.2)</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td>Itchiness at Site</td>
<td>113</td>
<td>45 (39.8)</td>
<td>60 (53.1)</td>
<td>8 (7.1)</td>
</tr>
<tr>
<td>Underarm Pain</td>
<td>113</td>
<td>98 (86.7)</td>
<td>12 (10.6)</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td>Underarm Swelling</td>
<td>113</td>
<td>106 (93.8)</td>
<td>6 (5.3)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Maximum (functional) Symptoms</td>
<td>113</td>
<td>30 (26.5)</td>
<td>θ (61.1)</td>
<td>14 (12.4)</td>
</tr>
</tbody>
</table>

B. Measurement Grading
Mild, <25mm; moderate, 25–50mm; severe, >50mm.

<table>
<thead>
<tr>
<th>Reactogenicity Parameter</th>
<th>DVC-LVS</th>
<th>USAMRIID-LVS</th>
<th>DVC-LVS Control</th>
<th>USAMRIID-LVS Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>N</td>
<td>None n (%)</td>
<td>Mild n (%)</td>
<td>Moderate n (%)</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>0</td>
<td>59 (52.2)</td>
<td>50 (44.2)</td>
</tr>
<tr>
<td>Induration</td>
<td>113</td>
<td>0</td>
<td>94 (83.2)</td>
<td>16 (14.2)</td>
</tr>
<tr>
<td>Maximum (measurement) Grade</td>
<td>113</td>
<td>0</td>
<td>58 (51.3)</td>
<td>50 (44.2)</td>
</tr>
<tr>
<td>Reactogenicity Parameter</td>
<td>USAMRIID-LVS</td>
<td>USAMRIID-LVS Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>-----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>None n (%)</td>
<td>Mild n (%)</td>
<td>Moderate n (%)</td>
</tr>
<tr>
<td>Erythema</td>
<td>115</td>
<td>0</td>
<td>71 (61.7)</td>
<td>40</td>
</tr>
<tr>
<td>Induration</td>
<td>115</td>
<td>0</td>
<td>101 (87.8)</td>
<td>12 (10.4)</td>
</tr>
<tr>
<td>Maximum (measurement) Grade</td>
<td>115</td>
<td>0</td>
<td>69 (60.0)</td>
<td>42 (36.5)</td>
</tr>
</tbody>
</table>
Table 2

Solicited Systemic Symptoms (Reactogenicity) by Symptom Type, Severity, and Study Group. The bottom row summarizes the number (percentage) of subjects overall who had no solicited systemic symptoms or, if solicited systemic symptoms occurred, the maximum severity experienced. Grading definitions as for functional grading in Table 1. A legend.

<table>
<thead>
<tr>
<th>Reactogenicity Parameter</th>
<th>DVC-LVS</th>
<th>USAMRIID-LVS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>None n (%)</td>
</tr>
<tr>
<td>Elevated Oral Temp.</td>
<td>113</td>
<td>110 (97.3)</td>
</tr>
<tr>
<td>Feeling Feverish</td>
<td>113</td>
<td>95 (84.1)</td>
</tr>
<tr>
<td>Sore Throat</td>
<td>113</td>
<td>86 (76.1)</td>
</tr>
<tr>
<td>Muscle Aches</td>
<td>113</td>
<td>88 (77.9)</td>
</tr>
<tr>
<td>Chills</td>
<td>113</td>
<td>102 (90.3)</td>
</tr>
<tr>
<td>Headache</td>
<td>113</td>
<td>73 (64.6)</td>
</tr>
<tr>
<td>Nausea</td>
<td>113</td>
<td>100 (88.5)</td>
</tr>
<tr>
<td>Feeling Tired</td>
<td>113</td>
<td>73 (64.6)</td>
</tr>
<tr>
<td>Joint Pain</td>
<td>113</td>
<td>102 (90.3)</td>
</tr>
<tr>
<td>Maximum Systemic Symptoms</td>
<td>113</td>
<td>45 (39.8)</td>
</tr>
</tbody>
</table>
Table 3
Numbers and Percentages of Positive Take Reactions at Vaccination Sites by Study Group A. Trial site investigators’ positive take assessments (physical examination). B. Take evaluation committee’s positive take assessments (digital photographs).

<table>
<thead>
<tr>
<th></th>
<th>DVC-LVS</th>
<th>95% CIs</th>
<th>USAMRIID-LVS</th>
<th>90% CIs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ITT</strong></td>
<td>N out of 113 (%)</td>
<td>81 (71.7)</td>
<td>62.4, 79.8</td>
<td>93 (81.6)</td>
</tr>
<tr>
<td><strong>PP</strong></td>
<td>N of 104 (%)</td>
<td>76 (73.1)</td>
<td>63.5, 81.3</td>
<td>88 (80)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>DVC-LVS</th>
<th>95% CIs</th>
<th>USAMRIID-LVS</th>
<th>90% CIs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IT</strong></td>
<td>N out of 112 (%)</td>
<td>103 (92)</td>
<td>85.3, 96.3</td>
<td>98 (87.5)</td>
</tr>
<tr>
<td><strong>PP</strong></td>
<td>N of 103 (%)</td>
<td>97 (94.2)</td>
<td>87.8, 97.8</td>
<td>94 (87)</td>
</tr>
</tbody>
</table>

Vaccine. Author manuscript; available in PMC 2018 August 24.