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# A Phase 1 clinical trial of a DNA vaccine for Venezuelan equine encephalitis delivered by intramuscular or intradermal electroporation

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ABSTRACT

Venezuelan equine encephalitis virus (VEEV), a mosquito-borne alphavirus, causes periodic epizootics in equines and is a recognized biological defense threat for humans. There are currently no FDA-licensed vaccines against VEEV. We developed a candidate DNA vaccine expressing the E3-E2-6K-E1 genes of VEEV (pWRG/VEEV) and performed a Phase 1 clinical study to assess the vaccine's safety, reactogenicity, tolerability, and immunogenicity when administered by intramuscular (IM) or intradermal (ID) electroporation (EP) using the Ichor Medical Systems TriGrid™ Delivery System. Subjects in IM-EP groups received 0.5 mg ($N=8$) or 2.0 mg ($N=9$) of pWRG/VEE or a saline placebo ($N=4$) in a 1.0 ml injection. Subjects in ID-EP groups received 0.08 mg ($N=8$) or 0.3 mg ($N=8$) of DNA or a saline placebo ($N=4$) in a 0.15 ml injection. Subjects were monitored for a total period of 360 days. No vaccine- or device-related serious adverse events were reported. Based on the results of a subject questionnaire, the IM- and ID-EP procedures were both considered to be generally acceptable for prophylactic vaccine administration, with the acute tolerability of ID EP delivery judged to be greater than that of IM-EP delivery. All subjects (100%) in the high and low dose IM-EP groups developed detectable VEEV-neutralizing antibodies after two or three administrations of pWRG/VEE, respectively. VEEV-neutralizing antibody responses were detected in seven of eight subjects (87.5%) in the high dose and five of eight subjects (62.5%) in the low dose ID-EP groups after three vaccine administrations. There was a correlation between the DNA dose and the magnitude of the resulting VEEV-neutralizing antibody responses for both IM and ID EP delivery. These results indicate that pWRG/VEE delivered by either IM- or ID-EP is safe, tolerable, and immunogenic in humans at the evaluated dose levels.

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## 1. Introduction

Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne alphavirus that causes periodic epizootics in the Americas, with infected equines serving as amplifying hosts [1]. Clinical symptoms of VEE may include fever, headache, vomiting, malaise and, rarely, encephalitis with somnolence [2]. Case-fatality rates for VEE are low (≤1%) [3]; however, VEEV is also recognized as a significant biological defense threat due to its ease of production, considerable stability, high infectivity through aerosol exposure, and low human infective dose (reviewed in [4]). As a result, VEEV has been classified as a Category B priority pathogen by both the Centers for Disease Control and Prevention and the National Institute of Allergy and Infectious Diseases.

There are currently no VEE vaccines licensed for use in the U.S. Although formalin-inactivated and live-attenuated vaccine candidates have been used in humans under Investigational New Drug (IND) status, the poor immunogenicity of the inactivated vaccine and the reactogenicity of the live vaccine have prompted studies on alternative approaches, including DNA vaccines [5–7]. DNA vaccines have shown promise in laboratory and early stage clinical studies for a variety of pathogens, and importantly for biodefense purposes, this platform has manufacturing and stability properties that are conducive to rapid production and efficient stockpiling [8].

We developed a candidate VEEV DNA vaccine candidate, pWRG/VEE, and have tested it in mice, rabbits and nonhuman primates (NHPs). When delivered by intramuscular (IM)

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electroporation (EP), pWRG/VEE elicited robust antibody responses, to include high levels of VEEV-neutralizing antibodies, in all three animal species and provided protection against aerosol VEEV challenge in mice and NHPs [9]. The VEEV-neutralizing antibodies elicited in rabbits also persisted at high levels for at least 6 months after vaccination.

Comparing IM- to intradermal (ID)-EP administration of pWRG/VEE in rabbits and NHPs demonstrated that both delivery methods elicited virus-neutralizing antibody responses of similar magnitudes, when normalized for dose. Moreover, similar levels of protection against aerosol VEEV challenge were elicited in NHPs receiving pWRG/VEE by IM- or ID-EP (Dupuy et al., manuscript in preparation). Here we report results of a Phase 1 study to assess and compare the safety, reactogenicity, tolerability, and immunogenicity of pWRG/VEE delivered at various doses by IM- or ID-EP.

## 2. Materials and methods

### 2.1. Vaccine and placebo

Construction of the pWRG/VEE DNA vaccine candidate expressing the E3-E2-6K-E1 genes of VEEV subtype IAB was described previously [9]. Briefly, codon optimization of the structural genes minus the capsid protein coding region (E3-E2-6K-E1), of VEEV IAB strain Trinidad donkey (Genbank accession number L01442) was accomplished using the GeneOptimizer™ bioinformatic algorithm followed by synthesis of the codon optimized genes (Geneart, Regensburg, Germany). pWRG7077 constructed by cloning the synthesized codon optimized genes into the *Not*I and *Bgl*II restriction sites of pWRG7077 (Supplemental Fig. 1).

Supplementary Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.04.077.

The vaccine plasmid was produced under current Good Manufacturing Practices (Althea Technologies, Inc., San Diego, CA) and vialed at 2.0 mg/ml in phosphate buffered saline. Flow-cytometry-based in vitro potency assays using a VEEV E1-specific monoclonal antibody to detect expression in transfected cells were performed as described earlier [10]. Subjects received the vaccine as vialed (high dose), diluted 1:3 in 0.9% sodium chloride injection, USP (Hospira, Inc., NDC 0409-4888-10) (low dose), or the diluent with no vaccine (placebo).

### 2.2. Electroporation delivery devices

The clinical use of the TDS-IM EP delivery device (Ichor Medical Systems, Inc., San Diego, CA) has been described [11]. For the TDS-ID device, the injectate was administered to the target tissue via needle-free jet injection (Medi-jector Vision, Antares Pharma) with distribution of the agent followed by the localized application of the EP inducing electrical fields with a 330 V/cm amplitude, 40 mS duration, and 10% duty cycle.

### 2.3. Overview of the clinical study design and enrollment of subjects

The study was sponsored by Ichor Medical Systems and conducted at Optimal Research (Accelovance), San Diego, CA, using a randomized, observer-blind, placebo-controlled, single-center design (IND # 015748). All recruiting and consent methods and materials were compliant with current Good Clinical Practice (GCP) guidelines and approved by the Aspire Institutional Review Board (IRB) http://aspire-irb.com and the Western IRB institutional biosafety committee was the Western IRB IBC (https://www.wirb.com/Pages/IBCServices.aspx). The subjects were prescreened by plaque reduction neutralization tests (PRNT) for the absence of neutralizing antibodies to VEEV as described previously [12] and were then randomized to receive either three doses of pWRG/VEE or three doses of placebo at days 0, 28, and 56 administered by either ID-EP or IM-EP. Final pWRG/VEE doses were 0.5 or 2.0 mg of DNA delivered by IM-EP and 0.08 or 0.3 mg delivered by ID-EP (Fig. 1).

### 2.4. Safety and immunogenicity

Safety was assessed at each dose administration (days 0, 28, and 56), at follow up visits two and 14 days after each dosing, and at study weeks 20, 32, and 52. Measurement of vital signs, assessment of injection site reactions, and a review of systemic reactions were performed at each study visit. Subjects were provided a memory aid, oral thermometer, and measuring device to assist in the daily documentation of any symptoms/local reactions occurring within 14 days of each dosing. Adverse events (AEs) were assessed by the investigator for severity and potential relationship to the vaccine candidate and/or administration procedure and each event was graded as: Grade 1 (mild, does not interfere with routine activities); Grade 2 (moderate, interferes with routine activities); Grade 3 (severe, unable to perform routine activities); Grade 4 (hospitalization or ER visit for potentially life-threatening event). Blood and urine samples were obtained at each follow up visit. Safety assays included albumin, sodium, potassium, glucose, bilirubin, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), complete blood count with differential, and urinalysis. Neutralizing antibodies against VEEV were measured for serum samples collected on days 0, 14, 42, 70, 140, 224, and 365 by PRNT as described previously [12].

### 2.5. Statistical methods

Descriptive analysis of safety and reactogenicity outcomes was performed for all subjects who received at least one dose of the DNA vaccine and for whom safety data were available. Summary tables were created in which incidence, intensity, and relationship to use of investigational product of individual solicited signs, symptoms, and other events were delineated by study group, severity, gender, and overall. Unsolicited AEs and serious AEs (SAEs) were analyzed in a similar fashion. For hematology and serum chemistry tests, any clinically significant change from baseline value was identified. The median, inter-quartile range and normal values for each of the laboratory values (as determined by the contract laboratory) were reported for each treatment group for each specimen collection point.

For the immunogenicity evaluation, the primary analyses variables were the proportion of seropositive subjects ($PRNT_{80} \geq 1{:}10$); overall rate of seroconversion; the magnitude of the immunological response; as well as the kinetics of the neutralizing antibody response and the duration of seropositivity. For each treatment group, a binomial proportion and exact 95% confidence interval (CI) were calculated. The secondary analysis variable is the geometric mean titers, with 95% CIs, of the $PRNT_{80}$ for VEEV-specific antibodies at each scheduled time point. Geometric mean titers, standard errors, and 95% confidence intervals (CIs) were calculated using log-transformed titers, replacing any titers below the limit of detection with 1.

## 3. Results and discussion

### 3.1. Clinical subject population and conduct of the study

The planned enrollment for this Phase 1 study included five randomized groups of 8 subjects each for a total of 40 subjects, with each administered a total of 3 injections of pWRG/VEE or placebo



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