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Phase II, dose ranging study of the safety and immunogenicity of single dose West Nile vaccine in healthy adults ≥50 years of age☆

Gustavo H. Dayan[a,*], Joan Bevilacqua[b], Dorothy Coleman[a], Aileen Buldo[a], George Risi[c]

[a] Sanofi Pasteur, USA, 1 Discovery Drive, Swiftwater PA 18370, United States
[b] Sanofi Pasteur, Canada, 1755 Steeles Ave West, Toronto, ON M2R 3T4, Canada
[c] Infectious Disease Specialists, PC, 614 West Spruce Street, Missoula, MT 59802, United States

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ABSTRACT

Introduction: ChimeriVax-WN02 is a live, attenuated chimeric vaccine for protection against West Nile virus (WNV) produced by insertion of the genes encoding the pre-membrane (prM) and envelope (E) proteins of WNV (strain NY99) into the yellow fever 7D vaccine virus. This Phase II, randomized, double-blind, placebo-controlled, multi-center study in the US assessed the immunogenicity, viremia, and safety of the ChimeriVax-WN02 vaccine.

Methods: The study included adults in general good health. Subjects aged ≥50 years were randomized to one of four treatment groups: ChimeriVax-WN02 4×10^3 plaque-forming units (pfu) ($n = 122$), 4×10^4 pfu ($n = 124$), 4×10^5 pfu ($n = 113$), or placebo ($n = 120$). A subset of subjects was randomized to assess viremia after vaccination at three different dose levels. Subjects were followed for safety up to 6 months after vaccination.

Results: A total of 121subjects for WN02 4×10^3, 122 for WN02 4×10^4, 110 for WN02 4×10^5, and 120 for the placebo group completed the study up to the 6-month safety follow-up. Seroconversion, as measured by plaque reduction neutralization test (PRNT), was achieved at Day 28 by 92.1%, 93.2%, and 95.4% of subjects in the WN02 4×10^3, the WN02 4×10^4, and the WN02 4×10^5 groups, respectively. Viremia was transient, detected between Days 2 and 14 but not at Day 28, and in most cases did not reach the quantification threshold. The percentage of subjects reporting at least one event of reactogenicity was similar in the placebo and active vaccine groups and showed no dose relationship.

Conclusions: The ChimeriVax-WN02 vaccine was highly immunogenic and well tolerated among subjects ≥50 years old at all dose levels.

1. Introduction

West Nile virus (WNV) of the genus *Flavivirus* can cause disease and death in horses, birds, and humans. When symptomatic, West Nile virus infection in humans typically causes a self-limited illness characterized by fever, headache, myalgia, rash, and anorexia, typically lasting 3–6 days [1]. Infection can progress to a central nervous system illness, which may be fatal or result in long-term morbidity [1,2]. Advanced age is a risk factor for severe disease [3]. Currently, a vaccine for the prophylaxis of WNV disease in humans is not available [4].

The first West Nile outbreak in the Western Hemisphere occurred in 1999 in New York City [5]. The virus further expanded in the US during the subsequent years. The incidence dropped to 0.2 per 100,000 in 2008 and further declined in 2009 [6]. However, in 2010 the number of West Nile neuroinvasive disease cases increased 62% from that reported in 2009 [7]. An increasing trend in the number of cases was also observed during the summer of 2012 [8]. Current prevention measures include personal protection and mosquito control, but vaccines could be an additional measure used in preventing illness and limiting outbreaks of West Nile virus infection [9].ChimeriVax™-WN02 is a live, attenuated chimeric vaccine candidate produced by insertion of the genes encoding the pre-membrane (prM) and envelope (E) proteins of WNV (strain NY99) into the yellow fever (YF) 17D vaccine virus [10]. The WNV E gene is mutated at three sites predicted to reduce neurovirulence, producing a highly attenuated phenotype. Preclinical studies demonstrated the protection of hamsters and mice against challenge with wild type (WT) WNV [11,12]. In young adult rhesus

Abbreviations: AE, adverse event; AR, adverse reaction; AUC, area under the curve; E, envelope; GMT, geometric mean titer; C_{max}, maximum concentration; PRNT, plaque reduction neutralization test; prM, pre-membrane; SAE, serious adverse event; SOC, system organ class; USP, United States Pharmacopeia; WNV, West Nile Virus; YF, Yellow Fever.

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* Corresponding author. Tel.: +1 570 957 2952; fax: +1 570 957 0934.
E-mail addresses: Gustavo.dayan@sanofipasteur.com (G.H. Dayan), Joan.Bevilacqua@sanofipasteur.com (J. Bevilacqua), GRisi@saintpatrick.org (G. Risi).

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macaques, ChimeriVax-WN02 induced neutralizing antibodies and protected against intracerebral challenge with WT WNV [12].

The ChimeriVax-WN02 vaccine was evaluated in a Phase I study in healthy volunteers aged 18–40 years where it was shown to be well tolerated and highly immunogenic [13]. The vaccine was plaque purified and was evaluated in a Phase II study [14], the first study to assess the safety, tolerability, viremia, and immunogenicity in healthy adults in different age cohorts, including the elderly. This study showed that ChimeriVax-WN02 vaccine was highly immunogenic in both younger and older adults and was well tolerated at all dose levels and in all age groups investigated. Levels and duration of viremia tended to be higher among older subjects, while peak viremia seemed similar in the age groups.

Since people aged older than 50 years are more likely to develop serious symptoms associated with WNV infections and represent the key population at risk [1,2], we conducted a study to further characterize the safety and immunogenicity profile of the ChimeriVax-WN02 vaccine in this age group. The objectives of this study were to assess the safety, viremia, and immunogenicity of three dose levels of ChimeriVax-WN02 vaccine among subjects ≥50 years of age.

2. Material and methods

2.1. Study design, population, and treatments

This randomized, dose-ranging, double-blind, placebo-controlled, multi-center study of the ChimeriVax-WN02 vaccine in healthy adults involved fifteen US centers in eleven states in the midwest, west, and south. Most centers were in states reporting WNV activity. The study was conducted from October 2008 to June 2009 in male and female subjects ≥50 years of age that were healthy or had medically-stable pre-existing illness or conditions and were living independently.

Subjects were randomized in blocks and stratified by site in a 1:1:1:1 ratio to one of four treatment groups: ChimeriVax-WN02 4×10^3 plaque-forming units (pfu), ChimeriVax-WN02 4×10^4 pfu, ChimeriVax-WN02 4×10^5 pfu, or placebo. A subset from each group was further randomized in a 2:1 ratio (viremia group:non-viremia group) to assess vaccine viremia. All subjects had a screening visit to determine study eligibility and received a single dose of ChimeriVax-WN02 vaccine or placebo on Day 0. Blood samples collected on Days 0 and 28 were analyzed for WN neutralizing antibodies. Additional blood samples were collected from subjects in the viremia subset on Day 0 and every other day until Day 14. Subjects were followed during the 28-day initial follow-up period post vaccination and for 6 months post vaccination.

The study was approved by the appropriate Institutional Review Board for each center and conducted in full accordance with Good Clinical Practice Consolidated Guideline approved by the International Conference on Harmonization and the ethical principles of the Declaration of Helsinki. Informed consent was obtained from each subject before they entered the study.

2.2. Vaccines

The lyophilized vaccine was supplied in 3 mL glass vials containing 4×10^4 pfu (Lot number WN04A13) and 4×10^5 pfu (Lot number WN04A08) vaccine virus and reconstituted in 0.5 mL 0.9% saline for injection, United States Pharmacopeia (USP). A vial containing the 4×10^4 pfu dose was serially diluted with ChimeriVax™ dose-ranging diluent to obtain the 4×10^3 pfu dose level. ChimeriVax dose-ranging diluent was supplied as a sterile solution of sugars, amino acids, salts, and human serum albumin, USP, at pH 7.5–8.5. The diluent did not contain preservative. The placebo was saline for injection, USP. Each 0.5 mL injection was administered subcutaneously in the deltoid region of the upper arm.

2.3. Immunogenicity

A plaque reduction neutralization test ($PRNT_{50}$) for ChimeriVax-WN02 vaccine was used to determine the neutralizing antibody levels. Serum was diluted from 1:5 to 1:10,240 and mixed with equal amounts of ChimeriVax-WN02 vaccine virus for final dilutions of 1:10–1:20,480. Neutralization was allowed to proceed over a 16 + 2 h period. This serum/virus mixture was then used to inoculate OrVax-Vero cells. Following the addition of an overlay, the infected cells were incubated for 5 days, then fixed and stained, and the plaques were counted. Titers were reported as the reciprocal of the dilution in which 50% of virus was neutralized.

Seroconversion was defined as a four-fold or greater rise in titer between pre- (Day 0) and post-injection (Day 28) samples.

Subjects who received a dose of study vaccine on Day 0, completed all study assessments, and had no significant protocol deviations were included in the immunogenicity analyses.

2.4. Vaccine viremia

Viremia was determined by plaque assay on duplicate Vero cell monolayers grown in 12 well plastic tissue culture plates. Viremia levels were defined as the average number of pfu/mL. Viremia was detectable at ≥20 pfu/mL and <60 pfu/mL and quantifiable at ≥60 pfu/mL. Blood samples were analyzed at Day 28 for subjects that were viremic on Day 14 to demonstrate the resolution of serum viremia. Duration of viremia was assessed by the number of days subjects showed viremia concentrations. The area under the curve (AUC) was calculated using the linear trapezoidal rule, beginning at Day 0 and using all non-missing scheduled viremia assessments, based on the serum viremia concentration at each scheduled time.

2.5. Safety and reactogenicity

2.5.1. Reactogenicity

Injection site reactogenicity (pain, erythema, and swelling) and systemic reactogenicity (fever, headache, malaise, and myalgia) were assessed Days 0 through 14 after vaccination. Intensity of pain, headache, malaise, and myalgia were graded as mild (no interference with activity), moderate (some interference with activity), or severe (significant, prevents daily activities). Intensity of erythema and swelling was graded by size as mild (2.5–5.0 cm), moderate (5.1–10 cm), or severe (>10 cm). Intensity of fever was graded by temperature as mild (≥38.0 °C to ≤38.4 °C), moderate (≥38.5 °C to ≤38.9 °C), or severe (≥39.0 °C).

2.5.2. Unsolicited adverse events (AEs) and adverse reactions (ARs)

Subjects were assessed for unsolicited AEs within the first 30 min after vaccination. Subjects recorded reactogenicity information and unsolicited AEs on a diary card from day 0 until the day 14 visit, and then only unsolicited AEs from Day 15 until the day 28 visit. Completed diary cards were collected at the day 14 and day 28 visits. AEs deemed related to vaccination were classified as ARs. Adverse events and reactions were graded as mild (no interference with activity), moderate (some interference with activity), or severe (significant, prevents daily activities).

2.5.3. Serious adverse events (SAEs)

Occurrence, nature, and outcome of SAEs were assessed throughout the trial and up to 6 months after vaccination. Subjects were to report SAEs immediately to the study center, which was