Phase II, Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Investigate the Immunogenicity and Safety of a West Nile Virus Vaccine in Healthy Adults

Rex Biedenbender,1 Joan Bevilacqua,2 Anne M. Gregg,*3 Mike Watson,4 and Gustavo Dayan3

1The Glennan Center for Geriatrics and Gerontology, Eastern Virginia Medical School, Norfolk; 2Sanofi Pasteur, Totonto, Ontario, Canada; 3Sanofi Pasteur, Cambridge, Massachusetts; and 4Sanofi Pasteur, Lyon, Lyonnais, France

Background. ChimeriVax-WN02 is a live, attenuated chimeric vaccine for protection against West Nile virus. This Phase II, randomized, double-blind, placebo–controlled, multicenter study assessed the immunogenicity, viremia, and safety of the ChimeriVax-WN02 vaccine.

Methods. The 2-part study included adults in general good health. In part 1, subjects aged 18–40 years were randomized to 1 of 4 treatment groups: ChimeriVax–WN02 3.7 \times 10^3 – 3.7 \times 10^4 plaque-forming units (PFU), 3.7 \times 10^4 PFU, 3.7 \times 10^3 PFU, or placebo. In part 2, subjects aged 41–64 and \geq 65 years were randomized to receive ChimeriVax-WN02 3.7 \times 10^2 PFU or placebo.

Results. In both part 1 and part 2, seroconversion was achieved at day 28 by \geq 96% of subjects in active treatment groups. In part 1, neutralizing antibody titers at day 28 were higher and viremia levels lower with the highest dose, whereas the adverse event profile was similar between the dose groups. In part 2, antibody titers and viremia levels were higher in subjects aged \geq 65 years, and more subjects in the 41–64 years cohort experienced adverse events.

Conclusions. The ChimeriVax-WN02 vaccine was highly immunogenic in younger adults and the elderly, and it was well tolerated at all dose levels and in all age groups investigated.

Clinical Trials.gov identifier: NCT00442169.

West Nile virus (WNV) is a virus of the genus Flavivirus that can infect humans, birds, mosquitoes, horses, and some other mammals. Infected humans may develop an illness that is characterized by fever, headache, backache, myalgia, and anorexia, typically lasting 3–6 days [1]. In addition, WNV may cause a severe illness involving the central nervous system, most commonly meningoencephalitis, which may be fatal or result in long-term morbidity [1]. Advanced age is the most important risk factor for neurological disease; people aged \geq 50 years are more likely to develop serious symptoms of WNV infection [1, 2]. WNV first appeared in the United States (US) in 1999 and subsequently spread across continental North America, the Caribbean, and South America [1-5]. Current methods for preventing WN infections are mosquito control and avoidance of mosquito bites [6]. There is no currently licensed vaccine for the prophylaxis of WNV disease in humans [6].

ChimeriVax-WN02 is a live, attenuated chimeric vaccine produced by insertion of the genes encoding the pre-membrane (prM) and envelope (E) proteins of WNV (strain NY99 [7]) into the yellow fever (YF) 17D vaccine clone [8]. The WNV E gene was later mutated at 3 sites predicted to reduce neurovirulence, producing a highly attenuated phenotype. Preclinical studies showed...
that this vaccine cannot be transmitted between mosquitoes [9]. Vaccination with ChimeriVax-WN02 protected hamsters and mice against challenge with wild type (WT) WNV [10, 11]. In young adult rhesus macaques, ChimeriVax–WN02 caused a transient viremia, induced neutralizing antibodies, and protected against intracerebral challenge with WT WNV [11]. A randomized, double-blind, placebo-controlled, Phase I study in healthy volunteers aged 18–40 years found that ChimeriVax-WN02 was well tolerated and highly immunogenic [12]. Most subjects experienced a transient low viremia; higher viremia levels were observed in subjects who received the lower dose of ChimeriVax-WN02.

ChimeriVax-WN02 has been further plaque-purified to generate a vaccine with an improved viremia profile. Here we report the immunogenicity, viremia, and safety results of the first Phase II study for ChimeriVax-WN02 in healthy young adults and the
first experience with ChimeriVax-WN02 in the elderly, which is the expected future target age group.

**SUBJECTS AND METHODS**

**Study Design, Population, and Treatments**

This randomized, double-blind, placebo-controlled, multicenter study of the ChimeriVax-WN02 vaccine in healthy adults involved 8 US centers. The study was done in 2 parts and included adults aged 18–40 years (part 1) or ≥41 years (part 2) in general good health with no history of vaccination against YF or Japanese encephalitis and no history of flavivirus infection.

In part 1, subjects were randomized to 1 of 4 treatment groups; ChimeriVax-WN02 3.7⁻¹⁰⁵ plaque-forming units (PFU), ChimeriVax-WN02 3.7⁻¹⁰⁴ PFU, ChimeriVax-WN02 3.7⁻¹⁰³ PFU, or placebo. The initiation of part 2 was contingent on a review of unblinded safety data by the Data Safety Monitoring Board (DSMB) and the US Food and Drug Administration (FDA). The ChimeriVax-WN02 3.7⁻¹⁰⁵ PFU dose was selected for part 2 on the basis of the analysis of the

---

**Figure 1.** Continued
immunogenicity, viremia, and safety data from part 1. Subjects were split into 2 age range cohorts, 41–64 years and >65 years; subjects in each age group were randomized to receive a single dose of ChimeriVax-WN02 3.7 \times 10^3 PFU or placebo in a staggered, age-ascending manner, allowing for review of safety data before larger numbers of subjects were treated. Twelve subjects in the 41–64 years cohort were initially given vaccine or placebo. After a favorable review of the unblinded adverse event (AE) and viremia data by the DSMB, the remaining 36 subjects in this group and the first 12 subjects in the >65 years cohort were given vaccine or placebo. Because no safety concerns were detected after a review of the unblinded AE and viremia data by the DSMB, the final 36 subjects in the >65 years cohort were given vaccine or placebo.

Each subject received a single dose of ChimeriVax-WN02 vaccine or placebo on day 0. Blood samples for WN neutralizing antibody analysis were taken on days 0, 14, 28, and 45, and at 6 months and 12 months after injection; samples taken on days 14 and 28 were split for immunoglobulin M (IgM) analysis. Blood samples for viremia studies were taken on days 1–14 and 21. Blood samples for St. Louis encephalitis (SLE) and YF neutralizing antibody testing were taken at screening. Solicited AEs were collected at clinic visits from days 1–14, 21, and 28; subjects completed diary cards from days 14–28.

Subjects in part 1 were allocated to treatment on the day of vaccination according to a prepared randomization schedule in the ratio 1:2:2:2 for placebo:ChimeriVax-WN02 3.7 \times 10^3 PFU:ChimeriVax-WN02 3.7 \times 10^4 PFU:ChimeriVax-WN02 3.7 \times 10^5 PFU. Randomized subjects were allocated the next sequential number and administered vaccine or placebo with the treatment supplies for that subject number. In part 2, subjects were stratified by age (41–64 years and >65 years) and assigned to vaccine or placebo in the ratio 1:2 for placebo:active vaccine. Subjects were blinded to treatment. All site personnel were blinded to the randomization scheme except for the study pharmacist, who prepared the dosing medication in syringes ready for administration. The DSMB was blinded to subject’s treatment assignment except at the prespecified time points of the planned safety analyses. An independent biostatistician designed the randomization scheme and did the unblinded analyses for the DSMB.

The study was approved by the appropriate institutional review board for each center and conducted in full accordance with the Good Clinical Practice Consolidated Guideline approved by the International Conference on Harmonization and the ethical principles of the Declaration of Helsinki. All subjects gave written informed consent before entering the study.

### Table 1. Summary of Subject Demographic Characteristics in Study of West Nile Virus Vaccine in Healthy Adults

<table>
<thead>
<tr>
<th>Part 1</th>
<th>Placebo (N = 17)</th>
<th>WN02 3.7 \times 10^3 PFU (N = 21)</th>
<th>WN02 3.7 \times 10^4 PFU (N = 37)</th>
<th>WN02 3.7 \times 10^5 PFU (N = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (52.9)</td>
<td>10 (47.6)</td>
<td>20 (54.1)</td>
<td>17 (60.7)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (47.1)</td>
<td>11 (52.4)</td>
<td>17 (45.9)</td>
<td>11 (39.3)</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>26.6 (6.4)</td>
<td>25.4 (5.6)</td>
<td>25.0 (6.4)</td>
<td>25.1 (6.5)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>25.0 (18–40)</td>
<td>24.0 (19–40)</td>
<td>24.0 (19–38)</td>
<td>22.5 (18–39)</td>
</tr>
<tr>
<td><strong>Race, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>16 (94.1)</td>
<td>19 (90.5)</td>
<td>35 (94.6)</td>
<td>26 (92.9)</td>
</tr>
<tr>
<td>African American</td>
<td>1 (5.9)</td>
<td>2 (9.5)</td>
<td>2 (5.4)</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n =</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;41–64 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>N = 11</td>
<td>WN02 3.7 \times 10^5 PFU N = 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1 (9.1)</td>
<td>8 (27.6)</td>
<td>2 (14.3)</td>
<td>9 (32.1)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (90.9)</td>
<td>21 (72.4)</td>
<td>12 (85.7)</td>
<td>19 (67.9)</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>49.9 ± 7.9</td>
<td>51.3 ± 6.0</td>
<td>70.4 ± 3.0</td>
<td>71.0 ± 4.8</td>
</tr>
<tr>
<td>Median (range)</td>
<td>49.0 (41–64)</td>
<td>50.0 (41–64)</td>
<td>70.0 (67–77)</td>
<td>70.0 (65–80)</td>
</tr>
<tr>
<td><strong>Race, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>11.0 (100)</td>
<td>22.0 (75.9)</td>
<td>14.0 (100)</td>
<td>28.0 (100)</td>
</tr>
<tr>
<td>African American</td>
<td>6 (20.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (3.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** N, no. of subjects in group; n, no. of subjects; PFU, plaque-forming units; SD, standard deviation; WN02, ChimeriVax-WN02.
Vaccines

The lyophilized vaccine was supplied in 3.0-mL glass vials containing $>1.5 \times 10^5$ PFU vaccine virus, reconstituted in 0.75 mL 0.9% saline for injection, United States Pharmacopeia (USP), then diluted with ChimeriVax dose-ranging diluent to the appropriate dose level. ChimeriVax dose-ranging diluent was supplied as a sterile solution of buffer salts, sugars, and human serum albumin, USP, at pH 7.5–8.5. The diluent did not contain preservative.

The placebo was saline for injection, USP, which was supplied in vials containing 2 mL sterile 0.9% sodium chloride. Each 0.5-mL injection was administered subcutaneously in the deltoid region.

Immunogenicity and Viremia Measurements

WN virus neutralizing antibodies were measured using a 50% plaque-reduction neutralization test (PRNT$_{50}$) against the homologous virus (ChimeriVax-WN02 vaccine) performed in Vero cells in 12-well plates [13]. After inoculation, cells were overlaid with methyl cellulose medium, incubated for 5 days at 37 °C, then fixed and stained using formalin solution and crystal violet. Plaques were counted using a microscope. SLE and YF neutralizing antibodies were also measured using a PRNT$_{50}$.

Figure 2. Seroconversion at day 28. All subjects had a prevaccination titer $<1:10$. In part 2, the day 28 antibody titer was not determined for 1 subject in the aged 41–64 years cohort and 1 subject in the aged $\geq 65$ years cohort. PFU, plaque-forming units; WN02, ChimeriVax-WN02.

IgM antibodies were measured qualitatively by an enzyme-linked immunosorbent assay (ELISA). Microtiter plates coated with WNV antigen were probed with dilutions of serum. Bound antibodies were detected with an anti-IgM enzyme conjugate; results were reported as negative, equivocal, or positive.

Viremia was determined by plaque assay on duplicate Vero cell monolayers. Viremia levels were defined as the mean number of PFU/mL. Viremia was detected using the crystal violet (negative stain) plaque technique in part 1, and a more specific positive staining technique (immunostain using a monoclonal antibody specific to the WNV envelope protein) was used in part 2.

Statistical Methods

The objectives of this study were to select a dose of ChimeriVax-WN02 vaccine for progression into an older age population after assessment of the safety, tolerability, viremia, and immunogenicity in healthy young adults aged 18–40 years; to evaluate safety, tolerability, viremia, and immunogenicity of a single dose of ChimeriVax-WN02 vaccine in healthy adult volunteers aged 41–64 years and $\geq 65$ years; to evaluate the duration of neutralizing antibody response in subjects receiving ChimeriVax-WN02 vaccine; and to investigate the immune response to ChimeriVax-WN02 vaccine in an elderly population.

**Immunogenicity.** Seroconversion rates were determined by a PRNT$_{50}$ assay and defined as a $\geq 4$-fold rise in titer between pre- and postinjection samples. Descriptive analyses of seroconversion rates, geometric mean titers (GMTs) of neutralizing antibodies, and IgM seropositivity rates were provided by treatment group (part 1) and by age range cohort (part 2). Seroconversion rates were compared at day 28. GMTs were analyzed at all available intermediate time points, and at 45 days, 6 months, and 12 months after vaccination in part 2 subjects. IgM seropositivity was presented at days 14 and 28 after vaccination.

The as treat per protocol (ATTP) population was used for immunogenicity analyses. The ATTP population included all subjects who were seronegative to ChimeriVax-WN02 at baseline, received a vaccination on day 0, had baseline and day 28 postvaccination blood samples for antibody analysis, and had no significant protocol deviations, but it included subjects who received the wrong treatment dose owing to a dosing error.

**Viremia.** A subject was considered viremic on a specific study visit if their viremia level on that day was detectable (ie, $>10$ PFU/mL). Vaccine viremia levels were summarized by study day and treatment group (mean viremia by study day and mean duration of viremia).

**Safety.** Safety analyses summarized the coded AE incidence and compared tabulated AEs between treatment groups 28 days postvaccination and at intervals between day 0 and day 28. Data were presented by dose levels for part 1 and by age range cohorts and combined in part 2. On the basis of clinical judgment, the investigator determined if an AE was related or unrelated to...
study treatment. Clinical laboratory and hematology results were summarized descriptively.

**Sample size and statistical analyses.** The number of subjects per group (n = 32) was designed to provide sufficient safety and tolerability data, particularly to identify common AEs occurring at a frequency of >10%. The rule of three was used for calculation of the sample size [14]. All statistical analyses were 2-tailed, assessed at the 5% significance level, and performed using SAS Version 9.1 (SAS Institute).

**RESULTS**

**Subjects Studied**
The study took place from 9 December 2005 to 27 July 2009. In part 1, there were 112 subjects. One subject in the ChimeriVax-WN02 3.7 × 10^5 PFU dose group was lost to follow-up, and all other subjects completed the study at least to day 28. A dosing error occurred at 1 study center, resulting in 8 subjects receiving the ChimeriVax-WN02 3.7 × 10^4 PFU dose instead of the ChimeriVax-WN02 3.7 × 10^3 PFU dose to which they were randomized. Therefore 31 subjects received the ChimeriVax-WN02 3.7 × 10^5 PFU dose, 40 received the ChimeriVax-WN02 3.7 × 10^4 PFU dose, 24 received the ChimeriVax-WN02 3.7 × 10^3 PFU dose, and 17 received placebo (Figure 1). Part 2 included 96 subjects; all subjects completed the study at least to day 28. Sixty-four subjects received the ChimeriVax-WN02 3.7 × 10^5 PFU dose and 32 subjects received placebo (Figure 1). In each part of the study, there were no major differences in demographics among the treatment groups (Table 1).

**Immunogenicity**
In part 1, seroconversion was achieved at day 28 by >96% of subjects in active treatment groups but none of the subjects in

---

### Table 2. Neutralizing Antibody Response (Geometric Mean Titer [GMT]) and Proportion of Subjects Positive for Immunoglobulin M after 1 Dose of WN02 Vaccine

<table>
<thead>
<tr>
<th>Part 1</th>
<th>Placebo (N = 17)</th>
<th>WN02 3.7 × 10^3 PFU (N = 21)</th>
<th>WN02 3.7 × 10^4 PFU (N = 37)</th>
<th>WN02 3.7 × 10^5 PFU (N = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutralizing antibody response, GMT (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 (before vaccination)</td>
<td>5.0 (5.0, 5.0)</td>
<td>5.0 (5.0, 5.0)</td>
<td>5.0 (5.0, 5.0)</td>
<td>5.0 (5.0, 5.0)</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.0 (5.0, 5.0)</td>
<td>16.8 (7.2, 39.5)</td>
<td>25.7 (18.3, 69.7)</td>
<td>26.9 (11.8, 61.5)</td>
</tr>
<tr>
<td>Day 28</td>
<td>5.0 (5.0, 5.0)</td>
<td>1367.3 (711.3, 2628.5)</td>
<td>2331.1 (1193.2, 4554.3)</td>
<td>3309.3 (1726.9, 6341.7)</td>
</tr>
<tr>
<td>IgM-positive subjects, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>0 (0.0)</td>
<td>5 (23.8)</td>
<td>22 (59.5)</td>
<td>10 (35.7)</td>
</tr>
<tr>
<td>Day 28</td>
<td>0 (0.0)</td>
<td>20 (95.2)</td>
<td>36 (97.3)</td>
<td>27 (96.4)</td>
</tr>
</tbody>
</table>

**Part 2**

<table>
<thead>
<tr>
<th>WN02 3.7 × 10^5 PFU</th>
<th>WN02 3.7 × 10^5 PFU</th>
<th>WN02 3.7 × 10^5 PFU</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥41–64 years</td>
<td>≥65 years</td>
<td>≥41 years</td>
<td>≥41 years</td>
</tr>
<tr>
<td>N = 29</td>
<td>N = 28</td>
<td>N = 57</td>
<td>N = 25</td>
</tr>
<tr>
<td>Neutralizing antibody response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean titer [95% CI]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 (before vaccination)</td>
<td>5.0 (5.0, 5.0)</td>
<td>5.0 (5.0, 5.0)</td>
<td>5.0 (5.0, 5.0)</td>
</tr>
<tr>
<td>Day 14</td>
<td>26</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>Day 28</td>
<td>15.3 (7.5, 31.4)</td>
<td>23.8 (11.9, 47.4)</td>
<td>19.2 (11.9, 31.2)</td>
</tr>
<tr>
<td>6 months</td>
<td>883.0 (362.1, 2153.1)</td>
<td>965.1 (442.3, 2105.8)</td>
<td>922.4 (519.2, 1638.5)</td>
</tr>
<tr>
<td>12 months</td>
<td>503.9 (194.6, 1304.8)</td>
<td>371.2 (130.1, 1059.1)</td>
<td>433.7 (218.5, 860.8)</td>
</tr>
<tr>
<td>IgM-positive subjects, n/M (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>10/27 (37.0)</td>
<td>16/28 (57.1)</td>
<td>26/55 (47.3)</td>
</tr>
<tr>
<td>Day 28</td>
<td>27/29 (93.1)</td>
<td>26/27 (96.3)</td>
<td>53/56 (94.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Antibody titer values reported as below the lower limit of quantification (LLOQ) of the assay were converted to LLOQ/2 (ie, values <10 were converted to 5). Antibody titer values reported as above the upper limit of quantification (ULOQ) of the assay were converted to ULOQ × 2 (ie, values >20480 were converted to 40960). CL, confidence limits; M, no. of subjects for the age cohort at the specified time; N, no. of subjects in group; n, no. of subjects; PFU, plaque-forming units; WN02, ChimeriVax-WN02.
Neutralizing antibody titers at day 28 were significantly different from placebo for all vaccine doses, and the GMTs were higher at higher doses. Among active vaccine recipients, antibody titers were substantially higher on day 28, compared with those on day 14 after vaccination, for each dose (Table 2).

In part 2, seroconversion was achieved at day 28 by 96% of subjects in both age cohorts in the active treatment group (Figure 2); none of the placebo subjects achieved seroconversion. In the active vaccine subjects, antibody titers at day 28 were consistently higher in subjects aged >65 years, compared with those of subjects aged 41–64 years, with the exception of the titers at day 45 (Table 2). However, these differences were not statistically significant because the confidence intervals overlap. Titers decreased in all age groups at subsequent time points after day 28, with the exception that the titer of subjects aged >65 years increased between day 45 and 6 months. Titers remained highest at 12 months among the ≥65 years cohort.

IgM Response

In part 1, an IgM response was observed in >95% of subjects in each active treatment group 28 days after vaccination; no response was seen in the placebo group. The percentage of subjects positive for an IgM response was substantially higher on day 28 (ie, >95%), compared with the corresponding percentage on day 14, for all active treatment groups (Table 2).

In part 2, 96.3% of subjects in the ≥65 years cohort and 93.1% of subjects in the 41–64 years cohort had an IgM response 28 days after vaccination; none of the subjects in any age cohort in the placebo group had an IgM response. In the active treatment group, the IgM response at day 14 was greater in the ≥65 years cohort than in the 41–64 years cohort (Table 2).

Vaccine Viremia

In part 1, the mean duration of viremic days was 3.9 days in the ChimeriVax-WN02 3.7 × 10^5 PFU dose group, 4.1 days in the ChimeriVax-WN02 3.7 × 10^4 PFU dose group, 4.8 days in the ChimeriVax-WN02 3.7 × 10^3 PFU dose group, and 1.4 days in the placebo group. Viremia levels tended to be inversely related to dose of vaccine. Viremia levels peaked around days 6 and 7 for the ChimeriVax-WN02 3.7 × 10^5 PFU group and on day 5 for the other 2 active treatment groups (Figure 3). In part 2, the mean duration of viremic days was 3.7 days in the 41–64 years cohort and 5.5 days in the ≥65 years cohort; 1 subject in the placebo group had detectable viremia. Viremia levels tended to be higher among the older subjects. Viremia peaked on day 6 for the ≥65 years cohort and around days 6 and 7 for the 41–64 years cohort (Figure 3).

Viremia generally decreased and reached zero by day 21 in subjects in all treatment groups in the study. There were no clear correlations between days of viremia and days of systemic reactions such as chills, malaise, headache, fever, and myalgia during the study.

Safety and Tolerability

The percentage of subjects with treatment-emergent AEs (TEAEs) in part 1 was highest in the placebo group (82.4%) and similar across the active treatment groups (range, 66.7%–71.0%). In part 2, 78.1% and 84.4% of subjects experienced TEAEs in the placebo and active treatment groups, respectively. More subjects in the 41–64 years cohort experienced TEAEs, compared with the ≥65 years cohort. In the 41–64 years cohort, TEAEs were reported by 93.3% and 93.9% of subjects in the placebo and active treatment groups, respectively. In the ≥65 years cohort, 64.7% and 74.2% of subjects in the placebo and active treatment groups, respectively, reported TEAEs. Most TEAEs were mild or moderate in severity.

The incidence of treatment-related TEAEs in part 1 was highest in the placebo group and lowest in the high-dose active treatment group (Table 3). In part 2, more subjects in the 41–64 years cohort experienced treatment-related TEAEs compared with the ≥65 years cohort. The incidence of treatment-related events in the 41–64 years cohort was greater among active treatment subjects, compared with placebo subjects (Table 3). In the ≥65 years cohort, the incidence of treatment-related TEAEs was similar between the treatment groups (Table 3).
Clinically significant laboratory abnormalities 28 days after active treatment were increased creatinine phosphokinase (CPK; 1 subject) and severely increased alanine aminotransferase (1 subject) in part 1, and decreased hemoglobin (1 subject); worsening anemia, decreased white blood cell count, and thrombocytopenia (1 subject); and elevated serum creatinine, glucose, and CPK (1 subject) in part 2. One subject experienced 4 serious AEs (SAEs); primary atypical pneumonia, acute cholecystitis, and 2 instances of chronic obstructive pulmonary disease. Other SAEs reported were cellulitis, obstructed abdominal hernia, and gastroenteritis. All SAEs were considered to be unrelated to study vaccine.

CONCLUSIONS

This is the first study to assess the safety, tolerability, viremia, and immunogenicity of ChimeriVax-WN02 vaccine in healthy adults in different age cohorts, including the elderly. The highest dose of the ChimeriVax-WN02 vaccine was selected for part 2 on the basis of results obtained in part 1, which showed that GMTs were higher and viremia levels were generally lower in this dose group, compared with the other dose groups, whereas the AE profile was similar between the dose groups. In part 2, antibody titers (with the exception of the day 45 titer) and viremia levels were higher in subjects aged >65 years, compared with those of subjects aged 41–64 years, although the differences in antibody titers were not statistically significant. More subjects in the 41–64 years cohort experienced TEAEs, compared with the corresponding number in the older cohort.

Neutralizing antibodies are one of the principal mediators of protective immunity against WNV infection [15]. High seroconversion rates were observed in our study (>96% in all dose groups and age groups assessed), consistent with the results of a previous clinical trial that assessed 2 doses of the ChimeriVax–WN02 vaccine (3.0 and 5.0 log10 doses) in 45 healthy adults [12]. In the previous study, seroconversion was 100% in both ChimeriVax–WN02 treatment groups on day 21 postvaccination. One subject (in the ChimeriVax–WN02 3.0 log10 treatment group) had a low titer that decreased and did not meet the definition of seroconversion on day 28; all other subjects who received the ChimeriVax–WN02 vaccine maintained seroconversion on day 28. In our study, there was a trend for increased neutralizing antibody titers with increased doses of vaccine. In contrast, the GMTs in the previous study were higher in the lower-dose group, compared with the higher-dose group [12].

In our study, antibody titers at day 28 were higher in subjects aged >65 years, compared with those of subjects aged 41–64 years. Titers dropped in all age groups at subsequent time points, remaining highest at 12 months among the cohort aged >65 years. In the previous study, 97% of ChimeriVax–WN02-vaccinated subjects were seropositive and retained high neutralizing antibody titers 12 months after vaccination [12].
Most subjects in our study experienced a transient low viremia, which was also observed in the previous study and is expected with YF and other chimeric vaccines [12]. As observed in the previous study, subjects who received the lower doses of ChimeriVax–WN02 vaccine had higher viremia levels, compared with those of subjects who received the higher doses. This paradoxical response may be due to a lower innate and delayed adaptive immune response to the lower dose of vaccine and has been observed with the YF 17D vaccine [16] and chimeric vaccine against Japanese encephalitis [17]. Viremia after YF 17D vaccination is followed by detectable levels of cytokines reflecting Toll-like receptor–mediated signaling [16, 18-20]. The mild systemic side effects of the YF 17D vaccine may be associated with this release of cytokines [20], and the strong and durable adaptive immune response to the YF 17D may be related to the robust innate immune responses to the vaccine [21].

The ChimeriVax–WN02 vaccine was well tolerated in our study. The most commonly reported TEAEs were fatigue, headache, myalgia, and other mild to moderate systemic reactions commonly associated with vaccines, as was observed in the previous study [12]. There was no clear correlation between viremia and systemic reactions during the study. There were no major differences in terms of safety between the younger and older age groups.

There were some limitations to our study. The sample size for each group may have not been large enough to obtain statistically significant differences among groups. Some false-positive viremia results were observed in the placebo group, particularly in part 1. This was mainly due to the assay used to detect viremia in part 1 (ie, crystal violet); in part 2 of the study, an immunostain technique was used, which was more specific and reduced the number of false-positives. Therefore, viremia was probably overestimated for part 1 of the study.

WNV was first detected in the Western Hemisphere in 1999 during an outbreak of human encephalitis in New York City [22]. In 2002 a dramatic increase in the number of cases was reported in the US with 4156 human cases, 284 deaths, and 15,257 equine cases [5, 23]. A larger epidemic occurred in 2003 with 9862 human cases and 264 deaths [5]. WNV spread not only within the United States but also in Latin America and the Caribbean [5].

Prevention efforts are based mainly on personal protection measures, such as repellent use, and mosquito control. However, if WNV continues to cause epidemic disease in the Americas, a safe and effective vaccine could be the optimal prevention tool [5]. Four WNV vaccines have been licensed for veterinary use in the US [11, 24-26], including a live attenuated WNV vaccine based on chimeric Flavivirus and very similar to the one used in this study [11]. Several approaches for the development of a vaccine in humans are also being investigated, including live attenuated vaccines [27], recombinant subunit vaccines [28-31], vectorized vaccines [32, 33], DNA vaccines [34], and live recombinant vaccines [12, 35], including the chimeric vaccine of this study.

In conclusion, in this study the 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. Further clinical studies to define vaccine safety and immunogenicity are warranted.

**Funding**

This work is supported by Sanofi Pasteur.

**Acknowledgments**

We thank the following principal investigators involved in this study: Jeffrey Gitt, Richard Greenberg, Steven Komjathy, Dennis Morrison, Richard Nathan, Greg Silver, and Miquel Trevino.

We thank Grace Mwawasi, for statistical support, and Melanie Lee of Dianthus Medical Limited, for assistance in the preparation of the manuscript on behalf of sanofi pasteur, in accordance with the European Medical Writers Association guidelines.

**References**