The Journal of Infectious Diseases

BRIEF REPORT

A Live Attenuated Chimeric West Nile Virus Vaccine, rWN/DEN4Δ30, Is Well Tolerated and Immunogenic in Flavivirus-Naive Older Adult Volunteers

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West Nile virus (WNV) is a major cause of mosquito-borne illness in the United States. Human disease ranges from mild febrile illness to severe fatal neurologic infection. Adults aged >60 years are more susceptible to neuroinvasive disease accompanied by a high mortality rate or long-lasting neurologic sequelae. A chimeric live attenuated West Nile virus vaccine, rWN/DEN4Δ30, was shown to be safe and immunogenic in healthy adults aged 18–50 years. This study evaluated rWN/DEN4Δ30 in flavivirus-naive adults aged 50–65 years and found it to be safe and immunogenic. Outbreaks of WNV infection tend to be unpredictable, and a safe and effective vaccine will be an important public health tool.

Keywords. West Nile virus; vaccine; flavivirus.

West Nile virus (WNV) is a positive-strand RNA virus of the family *Flaviviridae*, part of the Japanese encephalitis virus serocomplex that includes important human pathogens such as Murray Valley encephalitis, Japanese encephalitis, and St. Louis encephalitis viruses [1]. WNV has been present in Africa and Asia for decades and has usually been associated with mild illness that includes symptoms of low-grade fever, headache, rash, myalgia, and arthralgia. Recently, WNV has spread rapidly across the western hemisphere and is now the major vector-borne cause of viral encephalitis in the United States. By 2010, 3 million adults were estimated to have been infected with WNV in the United States, with nearly 13 000 cases of neuroinvasive disease, almost half of which occurred in adults >60 years of age [2–6]. In this age group, WNV infection can cause hepatitis, meningitis, and encephalitis, leading to paralysis, coma, and death. WNV is considered an emerging infection in the United States and presents a significant public health threat. This epidemiological trend of WNV suggests that the United States can expect periodic WNV outbreaks, underscoring the need for a safe and effective vaccine to protect at-risk populations, especially older adults.

The live attenuated WNV vaccine (rWN/DEN4Δ30) is a chimeric virus in which the prM and E structural protein genes of the DEN4Δ30 virus have been replaced by those of WNV strain NY99 [7]. Attenuation of rWN/DEN4Δ30 virus was achieved by 2 mechanisms: antigenic chimerization of WNV with nonneuroinvasive dengue virus serotype 4 (DENV-4) and the presence of a 30-nucleotide deletion in the 3′ untranslated region. This strategy has led to an acceptable balance of attenuation and immunogenicity, as demonstrated previously [7–12]. In nonhuman primates, rWN/DEN4Δ30 was highly attenuated, with no evidence of neuroinvasive disease, and all monkeys inoculated with a single dose of rWN/DEN4Δ30 demonstrated moderate-to-high levels of WNV-specific neutralizing antibodies and were completely protected against challenge with WNV NY99 [8, 9]. In addition, results of a comprehensive study of neuropathogenesis of the vaccine in the central nervous system of rhesus monkeys demonstrated a high level of neuro-attenuation of rWN/DEN4Δ30 as compared to that of the yellow fever 17D reference vaccine [10]. rWN/DEN4Δ30 also demonstrated restricted replication in young geese and reduced ability to infect, replicate, and disseminate in both *Culex* and *Aedes* mosquitoes, diminishing its risk of mosquito-bird-mosquito cycle transmission in wildlife [9, 11].

Previous studies evaluated rWN/DEN4Δ30 in humans in 2 separate clinical trials [12]. The vaccine lot WN/DEN4#1 (Novavax, Rockville, MD) was evaluated at 10^3 plaque-forming units (PFU) and 10^4 PFU in 56 healthy adult subjects between the ages of 18 and 50 years in phase 1 clinical trials at the Center for Immunization Research (CIR) of the Johns Hopkins Bloomberg School of Public Health (JHSPH; Baltimore, MD) and at Vanderbilt University (Nashville, TN). A separate study in 28 adult subjects evaluated a new vaccine lot, WN/DEN4#108A (manufactured by Charles River Laboratories, Malvern, PA), at a potency of 10^5 PFU given as 2 doses 6 months apart. The vaccine was well tolerated, safe, and immunogenic at all doses. No vaccinee developed a WNV-like illness, and there were no vaccine-related serious adverse events [12]. To assess safety and immunogenicity of this promising vaccine in a target population of older adults naive to WNV, we chose a dose of 10^4 PFU to perform a phase 1 trial of rWN/DEN4Δ30 in adults 50–65 years old.

Received 22 July 2016; accepted 13 October 2016; published online 25 October 2016.

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The Journal of Infectious Diseases® 2017;215:52–5

DOI: 10.1093/infdis/jiw501

METHODS

This study was conducted under an investigational new drug application (BB-IND no. 11940) at the CIR of the JHSPH and Robert Larner MD College of Medicine at the University of Vermont (UVMCOM; Burlington) and was approved by the institutional review boards and biosafety committees of both institutions. The National Institute of Allergy and Infectious Diseases Intramural Data Safety Monitoring Board was convened for periodic review of all study data. The trials were registered with Clinicaltrials.gov as NCT02186626.

Study Population

Twenty-eight healthy adult volunteers aged 50–65 were recruited from the Baltimore and Burlington areas. Informed consent was obtained prior to initiation of any study procedures. Individuals were eligible for inclusion if they were healthy, aged 50–65 years, and screen negative for hepatitis C and B viruses and HIV; had no evidence of prior DENV-1–4, WNV, St. Louis encephalitis virus, or yellow fever virus infection; and had normal screening values of liver function tests (LFTs), complete blood count (CBC), creatinine level, prothrombin and partial thromboplastin times, and urine analysis. Female subjects of childbearing potential were required to have a negative pregnancy test at screening and on vaccination day and were required to use a reliable method of contraception for the duration of the study. Subjects were also asked to be present for the duration of study, to complete a temperature card by recording oral temperatures 3 times daily, and to comply with study procedures of clinician examination and blood specimen collection.

Study Design

On the day of vaccination, subjects were randomly assigned to receive either 10^4 PFU of rWN/DEN4Δ30 or placebo (vaccine diluent) given as a single 0.5-mL subcutaneous injection. After vaccination, subjects were evaluated for reactogenicity and adverse events (Table 1) every other day through study day 16 and then on study days 21, 28, 56, 90, and 180. A blood specimen was collected at each visit through study day 28 for detection of viremia and/or performance of safety-associated laboratory analyses, including determination of a CBC with differential, determination of the alanine aminotransferase level, and coagulation studies. Neutralizing antibodies were measured against 3 viruses on days 0, 28, 56, 90, and 180.After administration of second dose or placebo at day 180, the laboratory and clinical assessments followed a schedule identical to the first-dose follow-up schedule. Neutralizing antibody was measured using the 50% plaque reduction neutralization test ($PRNT_{50}$) with 2 wild-type (WT) WNV strains (NY99-35262 and R94224) as the target viruses. Previously published genome sequence analysis of structural proteins indicated that R94224 belongs to the WN02 genotype of WNV [13]. Serum neutralizing antibody titers against vaccine virus (lot WN/DEN4#108A) were measured following receipt of the first and second vaccination. The durability of the antibody response was assessed 180 days following receipt of first and second vaccination. Viremia was assessed using a standard plaque-forming assay as described in previous studies [14]. Baseline characteristics and the frequency of adverse events (AEs) were compared between vaccine and placebo recipients. Statistical significance of the incidence of AEs was determined using the Fisher exact test, and for the mean viremia data, the Tukey-Kramer multiple comparison test was used (JMP, version 11.1.1; SAS Institute, Cary, NC).

Table 1. Summary of Systemic Adverse Events Following Administration of rWN/DEN4Δ30 Vaccine or Placebo

	Dose 1, Subjects, %			Dose 2, Subjects, %		
Adverse Event	Vaccine (n = 20)	Placebo (n = 8)	*P* Value	Vaccine (n = 19)	Placebo (n = 8)	*P* Value
Fever	0	0		0	0	
Headache	25	12.5	.639	26.3	25	.639
Rash	10	12.5	1.000	0.0	0	
Neutropenia	0	0		0	0	
Elevated ALT level	0	12.5	.286	0	0	
Myalgia	10	0	1.000	5.3	12.5	.513
Arthralgia	0	0		0	0	
Retro-orbital pain	0	0		0	0	
Fatigue	10	37.5	.123	10	25	.558
Nausea	25	12.5	.639	10.5	12.5	1.000
Photophobia	0	0		0	0	
Elevated PT	0	0		0	0	
Elevated PTT	5	0	1.000	0	0	

Abbreviations: ALT, alanine aminotransferase; PT, prothrombin time; PTT, partial thromboplastin time.

RESULTS

A total of 28 subjects were enrolled, with 14 women (50%) and 14 men (50%) between the 2 sites. At each site, 10 subjects received vaccine and 4 received placebo. The mean age of subjects was 55.5 years (range, 50–65 years). Eight subjects were aged ≥60 years. Eighteen subjects (64%) were white, and 10 subjects (36%) were African American. Vaccine and placebo recipients were distributed evenly between the male and female volunteers. There were no injection site reactions nor was fever reported in any subject. None of the observed AEs was significantly associated with vaccination. The most commonly reported AE was headache, in 5 (25%) of vaccinees and 1 placebo recipient (12.5%; Table 1). Two vaccinees and 1 placebo recipient experienced a rash similar to that associated with dengue vaccine; all cases were mild. Before receipt of the second dose, one of the vaccinees was incarcerated and was withdrawn from the study; the remaining 19 vaccinees received a second dose of vaccine at day 180. Of the 27 volunteers who received a second dose of vaccine or placebo, headache was again the

Table 2. Serologic Response to Wild-Type (WT) and Vaccine West Nile Viruses, by Time After Administration of WN/DEN4Δ30 Vaccine

Target Virus	Seroconversion,[a] Subjects, No. (%)	Geometric Mean Reciprocal $PRNT_{50}$ Titer Against WT and Vaccine Viruses			
		Peak After Dose 1 (Range)	Day 180 (Range)	Peak After Dose 2[b] (Range)	Day 360 (Range)
WN02	20 (95)	62 (19–274)	13 (<5–64)	26 (7–104)	20 (7–68)
WN99	20 (95)	66 (15–285)	28 (<5–194)	48 (8–126)	35 (5–103)
WN/DEN4Δ30	20 (95)	155 (64–286)	84 (38–134)	194 (95–296)	68 (36–122)

Abbreviation: $PRNT_{50}$, 50% plaque reduction neutralization test.

[a] Defined as a ≥4-fold rise in serum neutralizing antibody titer by day 90.

[b] Nineteen of 20 subjects received a second dose. Eighteen of 19 subjects had serologic findings from day 360, owing to incarceration of 1 subject.

most commonly reported AE, occurring in 3 vaccinees (26.3%) and 2 placebo recipients (25%; Table 1).

Vaccine viremia was detected in 3 subjects (15%) following receipt of the first dose; none of the subjects was viremic following receipt of the second dose. The peak viral titer was 0.7 $\log_{10}$ PFU/mL of serum, and viremia was detectable for a mean duration of 2.3 days.

The levels of neutralizing antibody against WNV were determined by $PRNT_{50}$, using WNV strain NY99 (WN99), which appeared in the United States in 1999; WN02 genotype strain (WN02), which was isolated from human brain tissue in 2008; or vaccine virus. The geometric mean peak titer for the 20 vaccinees following receipt of the first dose was 1:62, 1:66, and 1:155 when the target virus was WN02, WN99, and rWN/DEN4Δ30 virus, respectively (Table 2). For the 19 volunteers who received a second dose of rWN/DEN4Δ30, the geometric mean peak titer was 1:26, 1:48, and 1:194 against WN02, WN99, and rWN/DEN4Δ30 virus, respectively. Following receipt of the first dose of vaccine, 95% of vaccinees seroconverted ($PRNT_{50}$ titer, ≥ 1:10) by day 90.

DISCUSSION

Previous studies with rWN/DEN4Δ30 in adults aged 18–50 years demonstrated that the vaccine was safe, immunogenic, and well tolerated [12]. As the neurologic complications of WNV are highest in older adults, it is important to evaluate the performance and safety of rWN/DEN4Δ30 in this vulnerable age group, as they would be the ideal target age group for vaccination [4, 5]. The purpose of this study was to evaluate the immunogenicity and safety of rWN/DEN4Δ30 in that target population. Overall, the vaccine was well tolerated, and there were no significant differences in reported AEs between vaccine

Table 3. Neutralizing Antibody Titer Against WN/DEN4Δ30 Vaccine Virus, by Study Day, for Each Vaccinated Patient

Subject	Age at First Vaccination, y	Reciprocal $PRNT_{50}$ Titer Against Vaccine Virus							
		Day 28	Day 56	Day 90	Day 180	Day 208	Day 236	Day 270	Day 360
290.01.001	60	1320.0[a]	1182.0	561.0	487.0	279.1	433.8	814.6	679.1
290.01.002	54	159.0	220.0	237.0[a]	31.8	131.8	NA	NA	NA
290.01.003	61	141.0	164.0	178.0[a]	199.4	409.9	374.3	281.1	224.6
290.01.006	50	1310.0	1335.0	2633.0[a]	238.0	213.6	102.9	127.6	136.5
290.01.007	57	43.0[a]	15.0	4.0	5.2	173.7	167.7	139.3	19.9
290.01.008	62	4.0	461.0[a]	132.0	57.7	134.8	111.2	88.9	43.4
290.01.009	55	406.0[a]	137.0	113.0	24.2	144.7	111.9	41.3	50.8
290.01.011	51	5.0	486.0[a]	154.0	47.3	NA	NA	NA	NA
290.01.012	54	314.0	824.0[a]	546.0	161.5	248.9	128.6	119.6	104.0
290.01.013	57	220.0[a]	220.0[a]	116.0	39.1	24.8	22.4	25.7	16.7
290.01.015	54	80.7[a]	26.7	22.5	422.9	93.7	87.3	108.5	62.4
290.01.018	57	43.3[a]	42.4	35.4	286.8	73.4	102.8	148.6	135.0
290.01.019	52	182.8[a]	178.4	52.9	178.6	148.6	176.6	61.6	66.9
290.01.020	55	5.2	43.4	57.0[a]	100.8	54.3	128.0	43.5	46.6
290.01.021	52	144.4[a]	42.3	70.0	53.8	27.5	266.2	56.0	29.8
290.01.022	56	57.1	93.6[a]	50.6	147.9	68.0	720.2	143.3	109.6
290.01.023	56	55.9	84.0[a]	67.5	220.6	266.1	354.6	640.4	369.4
290.01.025	60	4.0	4.0	4.0	6.4	4.0	5.1	4.0	4.0
290.01.026	64	4.0	22.5	23.5[a]	16.9	41.7	26.3	69.4	19.4
290.01.028	53	4.0	6.4	10.3[a]	18.3	36.5	10.1	19.5	150.2
GMT (no. of values)	. . .	56.0 (20)	98.9 (20)	73.3 (20)	71.5 (20)	89.2 (19)	103.6 (18)	85.1 (18)	66.1 (18)

Abbreviations: GMT, geometric mean titer; NA, not available; $PRNT_{50}$, 50% plaque reduction neutralization test.

[a] Day of peak titer after initial vaccination.

recipients and placebo recipients. The most common AEs reported were headache and nausea in both vaccine recipients and placebo recipients. There were no neurologic manifestations, underscoring the apparent lack of neurotropism of rWN/DEN4Δ30, compared with WT WNV infection [9, 10]. This fact has been well documented in previous studies [12], but it is an important clinical finding to note, given the risk of West Nile neuroinvasive disease in adults >50 years old.

Following receipt of a single dose of rWN/DEN4Δ30, 19 of 20 vaccinees seroconverted to WT WNV, for an overall seroconversion rate of 95%. The ability of antibodies induced by the vaccine to neutralize WT WNV was evaluated against WN99, the prototypic strain of WNV, and the recent WN02 genotype virus. The WN02 genotype of WNV contains a characteristic, nonsynonymous Val-159-Ala substitution in the envelop protein that has been identified in the majority of WNV isolates since 2002 [13, 15]. The mean peak antibody titer against both WT viruses was not significantly different. The mean peak titers (reciprocal) against WN02, WN99, and vaccine rWN/DEN4Δ30 virus were 62, 66, and 155, respectively, after receipt of the first dose and 26, 48, and 194 following the second vaccination. Following receipt of the second dose of vaccine, a rise (≤4-fold) in the mean antibody titer, compared with the mean titer on study day 180, was noted for all 3 viruses. Although replicating virus was not detected in any subject following receipt of the second dose, the small increase in anamnestic response observed following the second vaccination suggests some viral replication below the assay's limit of detection (<0.5 $\log_{10}$ PFU/mL). The high rate of seroconversion following receipt of the first dose and the lack of 4-fold boost in antibody titer following receipt of the second dose suggest that a single dose of vaccine may be sufficient to induce a protective neutralizing antibody response. Neutralizing antibody titers at days 180 and 360 were similar. It appears that an acceptable balance of immunogenicity and attenuation is achieved with 1 dose of rWN/DEN4Δ30. The duration of this protection from a single vaccination is unknown, and further studies to examine the durability of neutralizing antibody may be useful in predicting immunization recommendations.

The results of this current study demonstrate that immunization with a single dose of rWN/DEN4Δ30 produces high levels of neutralizing antibody against WT WNV, inducing a 95% rate of seroconversion in an older population. This is an important observation, as age-related alterations in the immune system (ie, immunosenescence) may contribute to the increased susceptibility to infection and corresponding increase in disease among older adults. Clinically, the majority of severe infections with WNV occur in adults >60 years of age, thus rendering this age group an important target population for the vaccine. The current study reveals that, when given at a dose of 10^4 PFU, this vaccine is safe and well tolerated. The neutralizing antibody response seen in this older cohort was similar to what had been demonstrated in previous studies with younger adults, with individuals in each age group producing comparable mean peak antibody titers following receipt of one 10^4 PFU dose of rWN/DEN4Δ30 [12]. As there is currently no effective therapy for WNV infection other than supportive care, an effective vaccine may be key to reducing the incidence of severe neuroinvasive disease or death in older adults.

Notes

Acknowledgments. We thank R. Lanciotti (Centers for Disease Control and Prevention, Fort Collins, CO), for kindly providing both WNV strains; all of the dedicated volunteers and the UVMCOM Clinical Research Center and the Johns Hopkins University CIR and their excellent research nurses and staff, for their support; and the Office of Clinical Research Policy and Regulatory Operations, National Institutes of Health, for providing the regulatory oversight of this trial.

Financial support. This work was supported by the National Institute of Allergy and Infectious Disease Intramural Research Program, National Institutes of Health (contract HHSN272200900010C).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE form for potential conflicts of interest. Conflicts that the editors of the manuscript consider relevant to the content of the manuscript have been disclosed.

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