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The live attenuated chimeric vaccine rWN/DEN4Δ30 is well-tolerated and immunogenic in healthy flavivirus-naïve adult volunteers

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Abstract

WNV has become the leading vector-borne cause of meningoencephalitis in the United States. Although the majority of WNV infections result in asymptomatic illness, approximately 20% of infections result in West Nile fever and 1% in West Nile neuroinvasive disease (WNND), which causes encephalitis, meningitis, or flaccid paralysis. The elderly are at particular risk for WNND, with more than half the cases occurring in persons older than sixty years of age. There is no licensed treatment for WNND nor is there any licensed vaccine for humans for the prevention of WNV infection. The Laboratory of Infectious Diseases at the National Institutes of Health has developed a recombinant live attenuated WNV vaccine based on chimerization of the wild-type WNV NY99 genome with that of the live attenuated DENV-4 candidate vaccine rDEN430. The genes encoding the prM and envelope proteins of DENV-4 were replaced with those of WNV NY99 and the resultant virus was designated rWN/DEN4Δ30. The vaccine was evaluated in healthy flavivirus-naïve adult volunteers age 18 – 50 years in two separate studies, both of which are reported here. The first study evaluated 10^3 or 10^4 PFU of the vaccine given as a single dose; the second study evaluated 10^5 PFU of the vaccine given as two doses 6 months apart. The vaccine was well-tolerated and immunogenic at all three doses, inducing seroconversion to WNV NY99 in 74% (10^3 PFU), 75% (10^4 PFU), and 55% (10^5 PFU) of subjects after a single dose. A second 10^5 PFU dose of rWN/DEN4Δ30 given 6 months after the first dose increased the seroconversion rate 89%. Based on the encouraging results from these studies, further evaluation of the candidate vaccine in adults older than 50 years of age is planned.

Keywords

West Nile virus (WNV); live attenuated WNV vaccine; clinical trial

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INTRODUCTION

West Nile virus (WNV) is a member of the Japanese encephalitis virus serogroup of the genus *Flavivirus* belonging to the *Flaviviridae* family [1]. Humans are thought to be only incidental hosts in the transmission cycle of WNV in which birds serve as the amplifying hosts of the virus and *Culex* mosquitoes serve as the primary vector [1]. Although the majority of cases of WNV are asymptomatic, approximately 20% of infections result in symptomatic West Nile fever or neuroinvasive disease (WNND) manifesting as encephalitis, meningitis, or flaccid paralysis resembling poliomyelitis [2– 4]. The first outbreak of WNV in the Western Hemisphere occurred in New York in 1999, and since that time, WNV has become the leading vector-borne cause of viral encephalitis in the United States [1, 5]. By 2010, the number of adults infected with WNV in the U.S. was estimated to be nearly 3 million, with approximately 13,000 cases of WNND, almost half of which occurred in persons more than 60 years of age [6]. Importantly, the second largest recorded outbreak of WND in the United States occurred in 2012 with the CDC reporting 5,674 cases, including 2,873 (50.6%) of which were severe neurologic WNND and 286 deaths [7].

Economic analyses of WNV epidemics have demonstrated them to be costly [8, 9] and there is currently no licensed treatment or human vaccine for WNV. A low cost, efficacious vaccine may provide a cost-effective alternative for the prevention of WNV disease. Based on the success of the yellow fever and Japanese encephalitis vaccines, scientists at the Laboratory of Infectious Diseases have developed numerous live attenuated candidate flavivirus vaccines [10–15]. Many of these were evaluated in clinical trial and were demonstrated to be attenuated and immunogenic in adult flavivirus-naïve subjects [16–20]. A similar strategy was employed to develop a recombinant live attenuated chimeric WNV vaccine designated rWN/DEN4Δ30. The vaccine was highly attenuated for neurovirulence and neuroinvasiveness in mice compared with its wild-type parent virus WNV NY99 [21, 22]. Importantly, non-human primates immunized with a single dose of rWN/DEN4Δ30 were completely protected against challenge with wild-type WNV NY99 [21]. rWN/DEN4Δ30 also demonstrated reduced ability to infect, replicate, and disseminate in both *Culex* and *Aedes* mosquitoes, diminishing its risk of transmission from vaccinees to other hosts [23]. These data encouraged further evaluation of the rWN/DEN4Δ30 vaccine in healthy flavivirus-naïve adult subjects. Here we describe two Phase I clinical trials of rWN/DEN4Δ30 designed to examine the safety, immunogenicity, and dosing regimen of this promising candidate vaccine.

MATERIALS AND METHODS

Two studies of the live attenuated chimeric vaccine rWN/DEN4Δ30 were conducted under an investigational new drug application (BB-IND #11940) reviewed by the US Food and Drug Administration. The studies were conducted at the Center for Immunization Research (CIR) at the Johns Hopkins Bloomberg School of Public Health and the Vanderbilt University School of Medicine and were approved by the Institutional Review Boards and Biosafety Committees of both institutions. The National Institute of Allergy and Infectious Diseases (NIAID) Intramural Data Safety Monitoring Board was convened for periodic review of all study data. The trials were registered with Clinicaltrials.gov as NCT00094718 and NCT00537147.

Study population

Healthy adult male and non-pregnant female subjects were recruited from the Baltimore, MD and Nashville, TN metropolitan areas. Informed consent was obtained from each subject in accordance with the Code of Federal Regulations (21 CFR 50). Healthy subjects

between the ages of 18 and 50 years were enrolled if they met the following eligibility criteria: normal findings during physical examination; negative for antibodies to DENV-1, DENV-2, DENV-3, DENV-4, yellow fever, WNV, and St. Louis encephalitis viruses; negative for hepatitis B and C viruses; negative for HIV; normal values for complete blood count (CBC) with differential, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, creatinine phosphokinase, coagulation studies, and urinalysis. Female subjects were required to have a negative result on a urine pregnancy test at screening and on vaccination day and were required to use a reliable method of contraception.

Study design and clinical monitoring

Both studies were randomized, double-blind, placebo-controlled trials designed to assess the safety and immunogenicity of rWN/DEN4Δ30. Three doses of the rWN/DEN4Δ30 vaccine (10^3 PFU, 10^4 PFU, or 10^5 PFU), given as a single subcutaneous dose, were to be studied in a single trial. However, due to the limited availability of fully potent vaccine virus (see below), only the 10^3 PFU and 10^4 PFU cohorts were enrolled (Figure 1). After manufacture of a new vaccine lot, a second study was initiated to evaluate 10^5 PFU of rWN/DEN4Δ30, given at 0 and 6 months, (Figure 1).

On day of vaccination, subjects were randomly assigned to receive either vaccine or placebo (vaccine diluent) given as a single 0.5 ml subcutaneous injection. Volunteers were given a digital thermometer and a diary card to record their oral temperature three times per day for 16 days. After each vaccination, clinical assessments for local and solicited reactogenicity (Table 2) were performed every other day through study day 16 (and on day 19, single dose study) and again on study days 21, 28 and 42. Blood was drawn at each assessment for detection of viremia through study day 16 or 19 and for antibody assay on study days 0, 28, 42 and 180. After each vaccination, a CBC with differential, serum ALT, and coagulation studies were done at periodic intervals through study day 28. The clinical and laboratory assessments following the second dose of vaccine were identical to the first dose follow-up schedule. All adverse events were graded for intensity and relationship to vaccine as previously described [24].

Vaccine virus

rWN/DEN4Δ30 is a live attenuated chimeric virus vaccine produced using recombinant DNA technology and described previously [21, 22, 25]. The prM and E protein genes of the live attenuated DENV-4 vaccine candidate rDEN4Δ30 [26] have been replaced by those of the wild-type WNV strain NY99-35262 [25]. The remainder of the genome, including the capsid protein and all non-structural protein genes, is derived from the rDEN430 vaccine virus. Two rWN/DEN4Δ30 vaccine lots were generated and used in the two clinical studies. The first lot (WN/DEN4#1, titer of 3.1×10^5 PFU/ml) was produced and safety tested at Novavax, Inc. (Rockville, MD) with Good Manufacturing Practices (cGMP). The second lot (WN/DEN4#108A, titer of 2×10^7 PFU/ml) was produced and safety tested at Charles River Laboratories (Malvern, PA) with cGMP. The amino acid sequence of the polyprotein for these two vaccine lots did not differ [27]. Prior to administration, all vaccines were diluted to the appropriate titer with safety-tested L-15 medium (placebo). At each vaccination, aliquots of diluted and undiluted vaccine were titrated as described below to ensure potency of the vaccine administered.

Virus quantitation

The amount of virus in blood (viremia) was determined using a standard plaque- forming assay as previously described [26]. On the last day of detectable viremia for each subject, viral genomic RNA was isolated and reverse transcribed and a PCR cDNA fragment

corresponding to the end of NS5 gene and the 3′UTR of the DEN4-part of chimeric genome (nts 9735 – 10644; GenBank accession no. AY376438) was generated. A region (nts 9830 – 10434) around the Δ30 deletion in the virus genome was sequenced to evaluate the stability of the Δ30 mutation.

Serologic assessment

Serum neutralizing antibody titers against wt WNV strain NY99 were measured by the 60% plaque-reduction neutralization assay as previously described [21, 22]. Neutralizing antibody titer was defined as the highest dilution of serum that reduced the number of WNV plaques by 60% ($PRNT_{60}$). Seroconversion to WNV was defined as a ≥ 4-fold rise in serum neutralizing antibody titer to the wild-type WNV parent virus at either study day 28 or 42 post-vaccination, compared with the pre-vaccination $PRNT_{60}$. The durability of the antibody response was determined by measurement of the $PRNT_{60}$ at study day 180 following the first dose.

Data analysis

The purpose of this study was to describe the safety and immunogenicity of this novel vaccine candidate rather than to test formal statistical hypotheses. Secondary purposes were to determine the safety and immunogenicity of a booster dose of vaccine and to assess the safety profile of the different lots of the vaccine. Baseline characteristics and frequency of vaccine-related adverse events were compared between vaccine and placebo groups, between the subjects receiving one injection of vaccine and two injections of vaccine, and between subjects receiving the three different doses of the vaccine (10^3 PFU, 10^4 PFU and 10^5 PFU). Data from the two studies are presented together to improve the statistical validity of the results and because an important outcome of both studies was to determine which dose of vaccine (10^3 PFU, 10^4 PFU, or 10^5 PFU) should be further evaluated in older subjects. Statistical significance was determined using the Fishers exact test (adverse event data) or according to Tukey-Kramer multiple comparison test (viremia data) using JMP 7 software version 5.0.1.2 (SAS Institute, Cary, NC).

RESULTS

Demographics

A total of 212 subjects were recruited and 82 subjects were enrolled in the two studies (Figure 1). In total, 60 subjects received vaccine while 22 received placebo. Vaccinees ranged in age from 19 to 50 years while subjects receiving placebo ranged in age from 20 to 49 years. There was no statistically significant difference in mean age between subjects who received vaccine (31.3 years) and those who received placebo (31.6 years) (Table 1). There was also no statistically significant difference in mean age of subjects between the dose groups. There was no significant difference in the race or gender of vaccine recipients between dose groups and compared with placebo recipients. A total of 76 subjects were followed for the duration of the studies; six subjects were removed or withdrew from the study (Figure 1).

Vaccine virus

Undiluted and diluted vaccine was titrated on each vaccine day within 4 hours of vaccine virus removal from the −80°C freezer. For every vaccination except one, the titer of diluted vaccine virus was within 0.5 $\log_{10}$ PFU/ml of the target titer. However, when WN/DEN4#1 vaccine was prepared for 3 subjects in the 10^4 PFU cohort, the resulting titer of the undiluted vaccine was approximately 100-fold below the expected titer and the diluted vaccine was approximately 25-fold below the expected titer. Although these three subjects received a

dose of vaccine approximately $10^{2.6}$ PFU instead of the intended dose of 10^4 PFU, the safety and immunogenicity results have been included and will be commented on below.

Local reactogenicity and solicited adverse events

The vaccine was well tolerated by all vaccine recipients and no subjects experienced a systemic WNV-like or dengue-like illness. There was one serious adverse event that was unrelated to vaccine. A subject was hospitalized and diagnosed with diabetes mellitus 94 days after vaccination. There was no significant difference in the incidence of any solicited or local adverse event between vaccinees and placebo- recipients (Table 2) or by dose cohort (data not shown). The most commonly reported adverse events experienced by subjects were headache and rash. No subject experienced signs or symptoms of encephalopathy or meningitis. Three vaccine recipients and one placebo recipient developed fever following first vaccination. The fevers were due to inter-current illness, including acute influenza, and were determined to be unrelated or unlikely related to the vaccine. One vaccinee developed a fever following second vaccination. This fever was due to mild cholecystitis and was determined to be not related to vaccine. During physical examination it was noted that nine vaccine recipients and three placebo recipients developed a macular rash following first vaccination. The rashes were observed between 2 and 19 days following receipt of vaccine with a mean duration of 8 days. There were no rashes following revaccination. The rashes observed in the placebo recipients occurred on days 5 to 10 post-vaccination with a mean duration of 2.6 days.

There were no significant differences in the occurrence of abnormal laboratory values in vaccinees compared with placebo recipients. Four vaccinees and one placebo recipient developed transient mild neutropenia [defined as an absolute neutrophil count (ANC) of 1,000 – 1500/mm^3] lasting an average of four days. One vaccine recipient developed severe neutropenia (ANC nadir of 600/mm^3study day 6) and elevated CPK secondary to confirmed acute influenza B infection.

Viremia

Viremia was detected in eight vaccine recipients following first vaccination (Table 3). No subject developed detectable viremia after second vaccination. The peak viral titer (0.5 $\log_{10}$ PFU/ml) was at the lower level of detection and did not differ significantly by study or by vaccine dose. There was no significant difference in the onset or duration of viremia between cohorts. Virus isolates were prepared from serum collected from each vaccinee on the last day of detectable viremia (study day 8 to 19). Sequence analysis of these isolates revealed that the engineered Δ30 mutation remained unchanged in all virus isolates. Only one point mutation at nt 10308 (U → C) in the area adjacent to the Δ30 mutation was identified in one isolate.

Serological Response

Following one dose, 40 of 59 vaccinees (68%) seroconverted to wt WNV/NY99. The frequency of seroconversion by dose cohort ranged from 55% (10^5 PFU) to 75% (10^4 PFU) (Table 4). Two of the three subjects who received ~ $10^{2.6}$ PFU did not develop detectable neutralizing antibody against WNV NY99 however the third subject had detectable viremia following vaccination and developed a peak $PRNT_{60}$ of 1:155. Following a second dose of 10^5 PFU of vaccine, 5 of 7 (71%) subjects who had not seroconverted to wild-type WNV/NY99 following the first dose, seroconverted following the second dose. In addition, two vaccinees who had not met the definition of seroconversion by study day 42 did so by study day 180. Following two doses of vaccine, 89% of vaccinees met the definition of seroconversion (Table 5).

DISCUSSION

This vaccine candidate is a live chimeric flavivirus comprised of the prM and E protein genes of WNV NY99 and the capsid and non-structural protein genes of the attenuated DENV rDEN4Δ30. The vaccine is attenuated by two mechanisms: chimerization of WNV with a non-neuroinvasive flavivirus, DENV-4, and a 30-nucleotide deletion in the 3′UTR. Chimerization is a potent attenuation strategy and was the major factor that led to the satisfactory balance between attenuation and immunogenicity of rWN/DEN4 for mice and monkeys [21, 22, 25]. The advantage of a dual attenuation strategy is that the mutations are independently attenuating and make reversion to a wild-type WNV or DENV phenotype within a vaccinated host nearly impossible.

Overall, the vaccine was well tolerated with no significant differences noted in the occurrence of any systemic or local adverse event in vaccinees compared with placebo recipients. Importantly, there were no clinical signs or symptoms indicative of neurotropism of the vaccine in healthy adult volunteers. Following one dose of vaccine, the frequency of seroconversion against wild-type WNV NY99 was highest in the 10^3 and 10^4 PFU dose groups (74% and 75%, respectively) and lowest, surprisingly, in the 10^5 PFU dose group (55%), although these differences did not reach statistical significance. The rates of seroconversion to wild-type WNV induced by the WN/DEN4Δ30 vaccine were somewhat lower than that induced by another live attenuated chimeric WNV vaccine, ChimeriVax-WN02 [28]. However, the target virus used in the $PRNT_{50}$ assay for that vaccine trial was the immunizing vaccine virus itself and not wild-type WNV, a less stringent assessment. We found higher seroconversion rates and higher neutralizing antibody titers when WN/DEN4Δ30 was used as the target virus in the $PRNT_{60}$ assay (data not shown) but have presented the results of the assay with wild-type WNV as the target virus because we believe that it may be a more relevant assessment of immunogenicity.

Interestingly, two volunteers who received the 10^5 PFU dose of rWN/DEN4Δ30 and who had not seroconverted to WNV/NY99 by study 42, did seroconvert by study day 180. The first had a peak $PRNT_{60}$ of only 1:7 to WNV/NY99 on day 28 following first vaccination which increased to 1:22 on day 180 (prior to second dose). The second vaccinee did not have detectable antibody to WNV/NY99 by study day 42 following the first dose but developed a titer of 1:120 by study day 180. This volunteer received the first dose of vaccine on 9/10/08 and the second dose 3/11/09 and it is unlikely that the volunteer was exposed to WNV in the interim as it would have occurred during the very late fall period. Overall, 13/20 (65%) subjects who received the first 10^5 PFU dose of rWN/DEN4Δ30 seroconverted to WNV/NY99 by study day 180, which is comparable to that observed for recipients of 10^3 and 10^4 PFU of vaccine. We have observed a delayed development of peak antibody titer in some recipients of other chimeric flavivirus vaccines. In particular, 36% of recipients of a live attenuated tetravalent dengue vaccine containing a chimeric DENV-2 component developed peak titers to DENV-2 after study day 56. Unfortunately in our current study, serum was not collected between study day 42 and study day 180 and we therefore cannot establish the kinetics of the antibody responses in these two volunteers.

Only recipients of the 10^5 PFU dose received a second dose of vaccine and the overall frequency of seroconversion in this cohort improved to 89% following the second dose. Antibody titers were boosted following the second dose, increasing from a GMT of 1:15 at day 180 to 1:57 six weeks following second vaccination. It is unclear whether the observed antibody boost was secondary to undetected replication of the vaccine virus or caused by the antigen load delivered by the 10^5 PFU dose. Based on the evidence of boosting with a second dose of rWN/DEN4Δ30 given at 6 months, a second dose given at an earlier time-

point such as 3 or 4 months after the first dose may provide a boost in antibody titer and would be a more useful vaccination schedule.

A satisfactory balance between sufficient vaccine attenuation and immunogenicity can be difficult to achieve. Although rWN/DEN4Δ30 is highly attenuated, it was able to induce seroconversion to wild-type WNV/NY99 in 55% to 75% of vaccinees after a single dose. Higher seroconversion frequencies were observed with lower doses of the vaccine, something that has been demonstrated for other flavivirus vaccines [29–31]. The reduced viremia and immune response of volunteers who received the high dose of WNV vaccine might have been due to differences in the two manufacturing productions and subsequently, in accumulation of quasi-species and defective interfering particles, in particular, in the WN/DEN4#108 virus prepared at the higher titer. It should be noted that evidence for interference in high-dose infection of flaviviruses such as tick-borne Langat virus [29] and DENV-2 [32] was reported in monkeys and mice, respectively.

In summary, the rWN/DEN4Δ30 vaccine candidate was well tolerated and immunogenic. A single dose of 10^3 or 10^4 PFU of vaccine was able to induce seroconversion to WN/NY99 in ~75% of vaccinees. However, the titer of antibody required for protection against WND is not known and efficacy studies may be difficult because the incidence rate is low in the U.S. and outbreaks are sporadic. Because the majority of WNND occurs in persons older than 60, the target population for a vaccine would most likely be adults ≥ 50 years of age. Future studies are being designed to evaluate the safety and immunogenicity of rWN/DEN4Δ30 in adults older than 50. It will also be important to determine if a second dose of vaccine will improve either the seroconversion frequency or the durability of the antibody response and if so, establish the optimal interval between vaccinations.

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HIGHLIGHTS

- We evaluated two lots of a novel chimeric live attenuated WNV candidate vaccine in two Phase I clinical trials.
- The first trial evaluated 10^3 or 10^4 PFU of vaccine given as a single subcutaneous dose.
- The second trial evaluated a new lot of the vaccine given at a dose of 10^5 PFU with a booster dose given 6 months later.
- A single sub-cutaneous dose induced seroconversion to wild-type WNV NY99 in 55% – 75% of vaccinees.

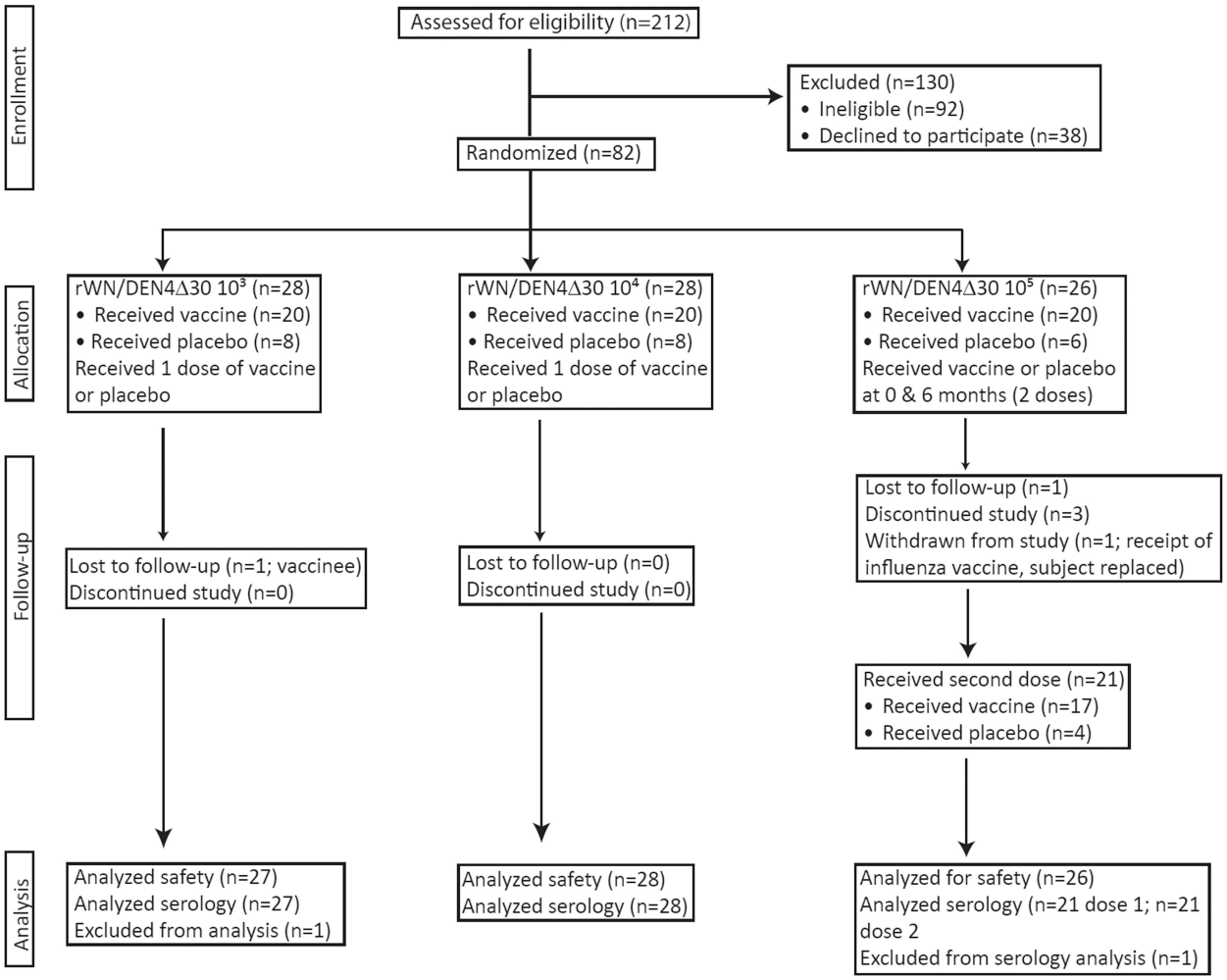

Figure 1.

Consort diagram illustrating the number of subjects screened, enrolled, and followed in the study as well as the number of subjects included in the safety and serologic analyses. A subject could be replaced if the immunogenicity data out to day 42 was not collected or could not be evaluated as defined by the protocol. Because one subject was replaced (a placebo recipient) a total of twenty-six subjects were enrolled in the 10^5 PFU cohort.

Table 1

Demographics of vaccine and placebo recipients

	10^3 PFU rWN/DEN4Δ30 (n=20)	10^4 PFU rWN/DEN4Δ30 (n=20)	10^5 PFU rWN/DEN4Δ30 (n=20)	Placebo (n=22)
Age ± SE (years)	29.4 ± 2.1	31.7 ± 2.1	33.1 ± 2.2	31.6 ± 1.7
Female (%)	55	40	75	68
Race (%)				
Black	45	20	70	50
Caucasian	50	75	30	45
Asian	5	5	0	0
Other	0	0	0	5

Table 2

Percentage of rWN/DEN4Δ30 vaccine (all dose levels) or placebo recipients who experienced indicated injection site or systemic adverse event[a]

Adverse event	rWN/DEN4Δ30 (n=60)	Placebo (n=22)	*p*-value (2-tailed)
Injection site:			
Erythema	1.7%	4.6%	0.47
Tenderness	3.3%	4.6%	1.00
Pain	3.3%	9.1%	0.29
Systemic[b]:			
Fever	5.0%[c]	4.6% [c]	1.00
Headache	25.0%	27.3%	1.00
Rash	15.0%	13.6%	1.00
Neutropenia[d]	6.7%	4.6%	1.00
Elevated ALT[e]	5.0%	4.6%	1.00
Elevated CPK	5.0%	9.1%	0.61
Fatigue	5.0%	13.6%	0.34
Myalgia	8.3%	4.6%	1.00
Arthralgia	1.7%	9.1%	0.17
Photophobia	1.7%	0.0%	1.00

[a] Only local and solicited adverse events that occurred are presented in the table. Other solicited and local adverse events that did not occur are not presented in the table for brevity.

[b] No subject experienced nuchal rigidity, neuropsychological, neurocerebellar, neuromotor, or neurosensory adverse events.

[c] Temperature elevation was determined to be unrelated to vaccine in all subjects.

[d] Absolute neutrophil count < 1,500/mm^3.

[e] Defined as a value > 1.25 above the clinical laboratory upper limit of normal.

Table 3

Summary of viremia in subjects vaccinated with rWN/DEN4Δ30 (first dose)

Vaccine candidate	Dose (log_{10} PFU)	No. subjects	No. infected[a] (%)	No. viremic (%)	Mean peak titer, log_{10} PFU/ml ± SE[b]	Mean day of onset of viremia ± SE[b]	Mean no. days of viremia ± SE[b]
rWN/DEN4Δ30	3.0	19[c]	14 (74)	3 (16)	0.5 ± 0.0	15.3 ± 1.9	2.3 ± 0.7
rWN/DEN4Δ30	4.0	20	15 (75)	4[d] (20)	0.5 ± 0.0	9.2 ± 1.8	3.2 ± 0.9
rWN/DEN4Δ30	5.0	20	11 (55)	1 (5)	0.5 ± 0.0	8.0 ± 0.0	1.0 ± 0.0

[a] Infection is defined as recovery of vaccine virus from the blood or a ≥ 4-fold rise in serum neutralizing antibody titer to vaccine virus at day 28 or 42 compared with day 0.

[b] Mean peak titer is calculated only for those subjects who were viremic.

[c] One subject withdrew from the study prior to the collection of samples for testing of viremia.

[d] One of these viremic subjects received only ~ $10^{2.6}$ PFU due to diminished potency of the vaccine

Table 4

$PRNT_{60}$ against WNV(NY99) induced by a single dose of rWN/DEN4Δ30 given at 10^3, 10^4, or 10^5 PFU

Vaccine candidate	Dose (log_{10} PFU)	No. subjects	Geometric mean $PRNT_{60}$ to WNV(NY99) (range)[a]				# sero-converting (%)[b]
			Day 0	Day 28	Day 42	Day 180	
rWN/DEN4Δ30	3.0	19	<5	64 (<5–1270)	161 (8–1530)	76 (<5–290)	14 (74)
rWN/DEN4Δ30	4.0	20	<5	117 (5–3218)	107 (<5–854)	35 (<5–232)	15 (75)
rWN/DEN4Δ30	5.0	20	<5	44 (18–183)	39 (9–330)	15[c] (<5–120)	11 (55)[d]

[a] Reciprocal titer. Geometric mean $PRNT_{60}$ calculated only for those subjects who seroconverted at study day 28 or 42.

[b] Defined as a ≥ 4-fold rise in serum neutralizing antibody against WNV(NY99) at day 28 or 42.

[c] 17/20 vaccinees returned for study day 180.

[d] Two vaccinees who did not have a ≥ 4-fold rise in serum neutralizing antibody titer by study day 42 did so by study day 180. These subjects were not included in % seroconversion per the protocol definition of seroconversion.

Table 5

A second dose of rWN/DEN4Δ30 at day 180 boosts the antibody response against WNV

Vaccine candidate	Dose (log_{10} PFU)	No. subjects	Geometric mean PRNT60 to WNV(NY99) virus (range)[a]			# sero-converting (%)[b]
			180	208	222	
rWN/DENΔ30	5.0	17	15 (<5–120)	39 (6–154)	57 (17–134)	15 (89)

[a] Reciprocal titer. Calculated for all subjects who seroconverted after first or second dose of rWN/DEN4Δ30.

[b] Defined as a ≥ 4-fold rise in serum neutralizing antibody against WNV(NY99) by day 222 compared with day 0.