Published in final edited form as:

Lancet Infect Dis. 2014 September ; 14(9): 830–838. doi:10.1016/S1473-3099(14)70811-4.

Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study

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See Online for appendix

Contributors

JEO, IDV, CT, AAH, CY-HH, MEB, and DTS conceived and designed the study. JS supervised clinical trial materials. IDV, CT, LL, AJ, MEB, and GSG undertook, supervised, or monitored the study. SS, JS, KLB, JLS, BEL, and JA obtained and analysed data. JEO, IDV, AAH, MEB, GSG, and DTS analysed and interpreted data. All authors contributed to the drafting of the report and approved the final submitted manuscript.

Declaration of interests

JEO, CT, AAH, SS, JS, JA, JS, GSG, and DTS were employees of Inviragen (now Takeda Vaccines)—the trial sponsor. CY-HH has a patent (Avirulent, Immunogenic Flavivirus Chimeras) licensed to Takeda Vaccines and with royalties paid to the Centers for Disease Control and Prevention (CDC), a patent (Compositions and Methods for Dengue Virus Chimeric Constructs in Vaccines) pending to Takeda Vaccines and CDC, and a patent (Compositions, Methods and Uses for Dengue Virus Constructs) pending to Takeda Vaccines and CDC. IDV, LL, AJ, KLB, JLS, BEL, and MEB declare no competing interests.

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Summary

Background—Dengue virus is the most serious mosquito-borne viral threat to public health and no vaccines or antiviral therapies are approved for dengue fever. The tetravalent DENVax vaccine contains a molecularly characterised live attenuated dengue serotype-2 virus (DENVax-2) and three recombinant vaccine viruses expressing the prM and E structural genes for serotypes 1, 3, and 4 in the DENVax-2 genetic backbone. We aimed to assess the safety and immunogenicity of tetravalent DENVax formulations.

Methods—We undertook a randomised, double-blind, phase 1, dose-escalation trial between Oct 11, 2011, and Nov 9, 2011, in the Rionegro, Antioquia, Colombia. The first cohort of participants (aged 18–45 years) were randomly assigned centrally, via block randomisation, to receive a low-dose formulation of DENvax, or placebo, by either subcutaneous or intradermal administration. After a safety assessment, participants were randomly assigned to receive a high-dose DENVax formulation, or placebo, by subcutaneous or intradermal administration. Group assignment was not masked from study pharmacists, but allocation was concealed from participants, nurses, and investigators. Primary endpoints were frequency and severity of injection-site and systemic reactions within 28 days of each vaccination. Secondary endpoints were the immunogenicity of DENVax against all four dengue virus serotypes, and the viraemia due to each of the four vaccine components after immunisation. Analysis was by intention to treat for safety and per protocol for

immunogenicity. Because of the small sample size, no detailed comparison of adverse event rates were warranted. The trial is registered with ClinicalTrials.gov, number NCT01224639.

Findings—We randomly assigned 96 patients to one of the four study groups: 40 participants (42%) received low-dose vaccine and eight participants (8%) received placebo in the low-dose groups; 39 participants (41%) received high-dose vaccine, with nine (9%) participants assigned to receive placebo. Both formulations were well tolerated with mostly mild and transient local or systemic reactions. No clinically meaningful differences were recorded in the overall incidence of local and systemic adverse events between patients in the vaccine and placebo groups; 68 (86%) of 79 participants in the vaccine groups had solicited systemic adverse events compared with 13 (76%) of 17 of those in the placebo groups. By contrast, 67 participants (85%) in the vaccine group had local solicited reactions compared with five (29%) participants in the placebo group. Immunisation with either high-dose or low-dose DENVax formulations induced neutralising antibody responses to all four dengue virus serotypes; 30 days after the second dose, 47 (62%) of 76 participants given vaccine seroconverted to all four serotypes and 73 (96%) participants seroconverted to three or more dengue viruses. Infectious DENVax viruses were detected in only ten (25%) of 40 participants in the low-dose group and 13 (33%) of 39 participants in the high-dose group.

Interpretation—Our findings emphasise the acceptable tolerability and immunogenicity of the tetravalent DENVax formulations in healthy, flavivirus-naive adults. Further clinical testing of DENVax in different age groups and in dengue-endemic areas is warranted.

Funding—Takeda Vaccines.

Introduction

Dengue virus circulates in nature as four distinct serotypes (1–4) transmitted mainly by the mosquito ***Aedes aegypti***. Dengue virus causes an estimated 390 million infections annually.[1,2] Infection can be either asymptomatic or have symptoms ranging from mild to life-threatening dengue haemorrhagic fever and dengue shock syndrome.[3, 4] Dengue fever is characterised clinically as an acute febrile illness with two or more symptoms that can include headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic features, or leucopenia.[5]

No vaccines or antiviral therapies are approved for prevention or treatment of dengue. Infection with one dengue virus serotype confers long-term protection against the homologous virus, but does not protect against infection with a heterologous virus. A second heterologous infection is associated with an increased risk of more severe disease,[6] whereas third or fourth infections rarely lead to disease.[7] Therefore, a vaccine against dengue virus needs to provide protective immunity against all dengue serotypes analogous to the response naturally induced after two or more sequential heterologous infections.[8]

DENVax is a tetravalent recombinant live-attenuated dengue vaccine based on a common virus backbone—a molecularly cloned, attenuated dengue virus serotype-2 strain,[9] termed DENVax-2.[10] In the tetravalent vaccine, chimeric viruses for serotypes 1, 3, and 4 were generated by substitution of the prM and E genes of DENVax-2 with those of serotypes 1, 3, or 4.[11,12] The safety and immunogenicity of tetravalent DENVax formulations have been

assessed in AG129 mice and non-human primates.[12,13] We did this phase 1 clinical trial to assess the safety, tolerability, and immunogenicity of two formu lations containing different ratios of tetravalent DENVax.

Methods

Study design and participants

We undertook a single-centre, double-blind, placebo-controlled, randomised, study between Oct 11, 2011, and Nov 9, 2011, in the town of Rionegro in the Antioquia region of Colombia. Because of its high elevation (2142 m above sea level), Rionegro is free of ***Aedes aegypti*** and dengue.

We enrolled healthy men and women aged 18–45 years who had normal findings during physical examination and were negative for antibodies to all dengue virus serotypes and to antibodies for yellow fever, West Nile virus, hepatitis B, hepatitis C, and HIV. Furthermore, eligible participants had to have normal values for complete blood count with differential, aspartate amino-transferase, alanine aminotransferase, creatinine, coagulation studies, and urinalysis. Women participants had to have a negative urine pregnancy test at screening and on each vaccination day, and those who were of childbearing potential were required to use an effective method of contraception and not be breastfeeding. Participants were ineligible if they had any immunodeficiency or chronic illness that could interfere with the study or if a flavivirus vaccination was planned during the trial. The study was designed to enrol 112 patients in four cohorts of 28 (23 to the vaccine groups and five to placebo). However, because of the population limitations in a rural community, we succeeded in enrolling only 96 patients. We deemed these numbers as sufficient for an exploratory, descriptive analysis of the safety of DENVax vaccines in healthy adults.

The clinical trial protocol was approved by the Ethics Committee at the Universidad de Antioquia and was done under an investigational new drug application with the US Food and Drug Administration and in accordance with the principles of the Declaration of Helsinki, Good Clinical Practices, and Colombian national regulatory requirements (Instituto Nacional de Vigilancia de Medicamentos y Alimentos [INVIMA]).

Randomisation and masking

Participants were recruited sequentially and randomly assigned (1:1), via block randomisation, first to two study groups given the low-dose formulation, either subcutaneously or intradermally. After initial safety analysis in this cohort, additional participants were recruited to receive the high-dose formulation, again in a 1:1 ratio, by either subcutaneous or intradermal administration. Within each group, participants were randomly assigned to receive DENVax vaccine or placebo (23:5). Group assignment was not masked from study pharmacists, but allocation was concealed from participants, nurses, and investigators.

Outcomes

Our primary endpoints were frequency and severity of injection-site and vaccine-associated systemic reactions, and frequency and severity of solicited and unsolicited adverse events. Secondary endpoints were geometric mean antibody titres and seroconversion rates to all four dengue virus serotypes, and incidence, duration, and titres for viral RNA for each of the four DENVax vaccine components after each administration.

Procedures

DENVax was manufactured with Vero cells in compliance with Current Good Manufacturing Practices by Shantha Biotechnics (Medchal, India).[14] Tetravalent DENVax was stored frozen at −60°C or lower. We used phosphate-buffered saline as placebo control. Two formulations of DENVax were given by subcutaneous (0·5 mL) or intradermal (0·1 mL) injections in the deltoid region, as two doses separated by an interval of 90 days. Low-dose formulations contained 8×10^3, 5×10^3, 1×10^4, and 2×10^5 plaque-forming units per dose of DENVax-1, DENVax-2, DENVax-3, and DENVax-4, respectively; high-dose formulations contained 2×10^4, 5×10^4, 1×10^5, and 3×10^5 plaque-forming units per dose, respectively. These doses were selected on the basis of non-human primate immunogenicity and challenge studies.[12,13] In these studies, doses of DENVax-1 and DENVax-2 that were greater than 1×10^3 plaque-forming units per dose provided complete protection from challenge, whereas DENVax-3 and DEVax-4 provided protection from challenge at all doses. DENVax-4 induced the lowest neutralising antibody titres; as such, both formulations contain more than 1×10^5 plaque-forming units per dose.

We assessed reactogenicity (solicited local and systemic reactions) and safety (unsolicited adverse events) at specified timepoints until day 120 after the first dose. Blood samples were collected at regular intervals for assessment of neutralising antibodies and viraemia against all four virus serotypes.

Local or systemic adverse events over the 28 day period after the first and second dose of vaccination were recorded both on a diary card (solicited adverse events) and through investigator assessment (unsolicited events). Solicited local reactions assessed included pain, redness and swelling, and itching at the injection site. Systemic solicited reactions monitored included fever, headache, malaise, myalgia, eye pain, abdominal pain, and arthralgia. We documented and followed up serious adverse events that happened any time during the study.

We measured titres of anti-dengue-virus antibody in sera collected on days 0, 15, 30, 60, and 90 after the first immunisation and on days 14 and 30 after the second immunisation with a 96-well immunofocus-based plaque- reduction neutralisation test (PRNT) in accordance with guidelines from WHO.[15] The neutralising antibody assay was done as previously described[13] with use of wild-type viruses from which the prM and E genes of each DENVax virus were derived (sereotypes 1 [strain 16 007], 2 [16 681], 3 [16 562], and 4 [1036]).[11] The immunostained viral foci were then quantified with an ELISpot reader (AID Instruments, Strassberg, Germany) and the viral plaque count was used to identify the lowest serum dilution that resulted in reduction of greater than 50% in the input plaque values

($PRNT_{50}$). Samples with $PRNT_{50}$ of less than 10 (the reciprocal of a 1:10 dilution and the lower limit of quantitation) were deemed seronegative and assigned a $PRNT_{50}$ value of 5 for calculation of the geometric mean titres. We defined seroconversion as either an initially seronegative patient who became seropositive after vaccination, or a four-fold or greater increase in titre in initially seropositive patients.

DENVax viral RNA was measured by quantitative RT-PCR with primers specific for each of the DENVax-1, 2, 3, and 4 components, as previously described,[12,16] in sera collected on days 0, 2, 4, 5, 7, 9, 11, 15, and 30 after the first and second immunisations. Results were expressed as $\log_{10}$ genome equivalents per mL (GE/mL) of serum. The limit of quantitation for the RT-PCR assays for DENVax-1–3 was 3·6 $\log_{10}$ GE/mL of serum; the limit for DENVax-4 was 3·9 $\log_{10}$ GE/mL. Samples that were positive for the presence of viral RNA were then tested by viral plaque titration directly on Vero-cell monolayers for the presence of replication-competent vaccine virus. To assess genetic stability, samples containing infectious vaccine viruses were subjected to spot sequencing of the three attenuating loci present in each of the DENVax constructs, as described previously.[14]

Clinical laboratory data, including haematology, chemistry, liver function tests, and urinalysis, were done locally on samples obtained before dosing and on days 7, 15, 21, 90, 97, 104, and 111 after the first injection. We compared changes from baseline values to identify shifts in severity grade with treatment. We also compared the mean values and changes from baseline over time.

Statistical analysis

We summarised antibody responses with geometric mean titres and 95% CIs. We summarised clinical findings and incidence with number and percentages. Analyses were done with actual results and no missing data were imputed. Because of the small sample sizes in this study, no detailed statistical comparisons of adverse event rates were warranted. Analysis was by intention to treat for safety and and per protocol for immunogenicity. The trial is registered with ClinicalTrials.gov, number NCT01224639.

Role of the funding source

Takeda employees and subcontractors (through a contract research organisation) had a role in study design, data collection, data analysis, data interpretation, and writing of the report. All study data were available to all authors upon request. JEO and DTS had final responsibility for the decision to submit for publication.

Results

We randomly assigned 96 patients to one of the four study groups (figure 1): 40 participants (42%) received low-dose vaccine and eight (8%) received placebo in the low-dose groups; 39 (41%) received high-dose vaccine, with nine (9%) assigned to receive placebo. Five patients (10%) from the high-dose groups withdrew from the study before receiving the second dose: four withdrew voluntarily because they moved out of the study area and one withdrew because of fever immediately before the second dose (figure 1). 70% of the study population were women (table 1). The median age of participants was 20 years (range 18–

43) for the vaccine group and 21 years (18–45) for the placebo group. 73 (92%) of 79 participants were mestizos and the remaining six participants (8%) were white, distributions that are representative of the Colombian population overall.

Most solicited and unsolicited adverse events were mild or moderate (table 2). We noted no cases when patients had two or more adverse events consistent with dengue including fever, photophobia, muscle pain, joint pain, or rash (appendix). The incidence of fever or rash was low and reported with similar frequency in the vaccine and placebo groups (appendix). We recorded two serious adverse events, which were not associated with the vaccination: a suicide attempt 3·5 months after the second dose in a participant in the placebo group, and a fractured ankle 2 months after the second dose in a patient receiving high-dose subcutaneous vaccine. We noted no clinically meaningful differences in the overall incidence of systemic adverse events between patients in the vaccine and placebo groups (table 2). Headache and fatigue were the most commonly reported systemic adverse events after the first and second doses combined (appendix). By contrast, local solicited reactions were more common in patients receiving vaccine than in those receiving placebo (table 2). Intradermal vaccination led to a numerically higher incidence of local reactions than did subcutaneous administration (20 [95%] of 21 participants in the low-dose and 18 [100%] of 18 participants in the high-dose intradermal groups compared with 15 [79%] of 19 and 14 [67%] of 21 participants, respectively, in the subcutaneous groups; appendix). Injection-site erythema (redness) was the most commonly reported local solicited adverse event (appendix). Assessment of the concomitant drugs showed no clinically meaningful differences between participants in the vaccine versus placebo that would be indicative of other adverse events (data not shown).

No clinically relevant changes took place in white-blood-cell, neutrophil, or platelet counts, or concentrations of aspartate aminotransferase or alanine aminotransferase after vaccination, and there were no significant differences in these measures between patients in the vaccine and placebo groups (appendix).

The number of patients who tested positive for viral RNA, and the quantity of DENVax viral RNA detected, were similar after intradermal or subcutaneous administration (data not shown). 17 (43%) of 40 participants in the low-dose vaccine group and 33 (85%) of 39 participants in the high-dose group tested positive for viral RNA to any one of the DENVax components after the first administration. Table 3 shows the number of participants with detectable vaccine viral RNA by day after the first dose. No viral RNA was detected immediately after vaccination (days 0–4) and viral RNA was most often detected on days 9 and 11 (table 3). All participants were negative for viral RNA on day 30, showing clearance of the vaccine. DENVax-1 viral RNA was not detected in any of the participants in either the low-dose or high-dose cohorts. DENVax-2 was the most commonly detected viral RNA in both cohorts, identified between days 7 and 15 in the low-dose group and days 5 and 15 in the high-dose group (table 3). After the first administration, DENVax-3 viral RNA was detected in three participants (4%) between days 5 and 9 (one in the low-dose group and two in the high-dose group), whereas DENVax-4 viral RNA was identified in only one (1%) participant in the low-dose group on day 7.

Replication of DENVax viral RNA was less common after administration of the second vaccine (data not shown). Only four (5%) of 76 participants were positive: one individual was positive for DENVax-2 RNA 7–14 days after the second administration (this individual failed to seroconvert to any serotype after the first administration); one individual was positive for DENVax-3 at day 11; and two were positive for both DENVax-3 and DENVax-4 RNA, one on day 7 and one on day 11. In these four cases, viral replication was short in duration at low levels (3·9–4·3 GE/mL).

Infectious DENVax viruses were detected in only ten (25%) of 40 participants in the low-dose group and 13 (33%) of 39 participants in the high-dose group. 22 (28%) participants tested positive for DENVax-2 (nine in the low-dose group and 13 in the high-dose group), two of whom were also positive for DENVax-3; one participant was positive only for DENVax-4. The observed viraemia was of short duration (most were positive for only one sampling day) and the titres ranged between 10 and 430 PFU/mL (table 3).

DENVax isolates from all participants with viraemia were partly sequenced. The attenuating mutations in the NS1 and NS3 genes (present in all individual DENVax constructs) were intact in all infectious viruses isolated after vaccination. For the 5′ NCR-57 attenuating locus, reversion was detected in viruses isolated from nine participants (11%): four from the low-dose group and five from the high-dose group.

Administration of DENVax induced detectable neutralising antibody responses to each of the four serotypes. For both routes and formulations, the highest induced geometric mean titres were to dengue virus serotype 2 (figure 2). High neutralising antibody titres were also detected to serotypes 1 and 3. Serotype 4 had the lowest neutralising antibody titres (figure 2). For both routes and formulations the second administration did not substantially increase antibody titres against virus serotype 2, but slight increases in titre were recorded for serotypes 1, 3, and 4 at day 120 versus day 90 (figure 2). There were no significant differences in neutralising antibody titres between the groups because of the small sample sizes (data not shown).

After administration of the first dose, levels of DENVax seroconversion ranged between 24% and 100% for each serotype (table 4). After two doses, the highest rates of seroconversion were consistently noted for serotypes 1 and 3, followed by serotype 2, and then serotype 4 (table 4). Notably, the high-dose formulation induced seroconversion to serotypes 1–3 in more than 90% of participants and seroconversion to serotype 4 in 47–77% of participants (table 4).

One dose of DENVax induced seroconversion to two or more dengue viruses in 84–100% of participants (figure 3). Seroconversion to three or more viruses was higher in participants in the high-dose group (17 [81%] of 21 receiving subcutaneous vaccine and 17 [94%] of 18 receiving intradermal vaccine) than in those in the low-dose group (13 [68%] of 19 and 15 [72%] of 21, respectively). After one dose, seroconversion to all four dengue virus serotypes (tetravalent responses) was noted in five (23%; low-dose, subcutaneous) to 11 (61%; high-dose, intradermal) participants in the vaccine groups on day 30 (figure 3).

A second dose of DENVax increased the overall multivalent responses. Responses to two or more viruses were noted in 18 (95%) to 21 (100%) of the participants in the DENVax groups (figure 3), with 17 (90%) to 19 (100%) showing responses to three or more virus serotypes. Tetravalent responses also increased with the second dose (figure 3): 11 participants (58%) seroconverted to all four viruses in the low-dose, subcutaneous group; 15 (71%) in the low-dose, intradermal group; nine (47%) in the high-dose, subcutaneous group; and 12 (71%) in the high-dose, intradermal group.

Panel: Research in context

Systematic review

On April 28, 2014, we searched PubMed for reports published at any time previously, and written in English, with the term “dengue vaccine clinical trial”. The search revealed 38 research articles describing clinical trials. Previously published clinical trials that assessed dengue vaccine candidates included the testing of monovalent, bivalent, trivalent, and tetravalent mixtures of live attenuated viruses,[23–34] recombinant live attenuated viruses,[35–45] or DNA vaccines.[46] Early efforts by Mahidol University–SanofiPasteur or Walter Reed Army Institute of Research–GlaxoSmithKline using tetravalent mixtures of live attenuated viruses have been suspended because of difficulties in balancing attenuation with immunogenicity for four different viruses.[28,31,32,34,47,48]

Three different recombinant attenuated virus approaches are in clinical trials. Sanofi Pasteur has completed and published several phase 1 and 2 clinical trials, characterising the safety and immunogenicity of a tetravalent chimeric recombinant dengue vaccine based on the yellow-fever backbone.[36,37,41,42,45] This vaccine was recently tested in a phase 2b efficacy trial[21] in Thailand and showed 30% protection against dengue fever; the vaccine did not protect against dengue caused by infection with serotype 2, which caused most the cases in this single-centre trial.

Various clinical trials of monovalent dengue viruses containing a 30 nucleotide deletion in the 3′ untranslated region have been done by investigators at the National Institutes of Health and Johns Hopkins University.[35,38,39,43,44,49,50] These investigators have assessed tetravalent combinations of these recombinant viruses by mixing monovalent vaccines at the clinical site.[40] A prototype monovalent DNA vaccine has been assessed in a phase 1 clinical trial.[46]

Interpretation

This is the first report of the safety and immunogenicity of a tetravalent recombinant dengue vaccine that is based on a dengue serotype-2 backbone. Use of this backbone is highly relevant in view of the failed protection against this serotype by the dengue vaccine based on chimeric yellow fever. Both high-dose and low-dose vaccine formulations of the four DENVax components were well tolerated, with mostly mild and transient local or systemic reactions. Immunisation with either formulation induced significant neutralising antibody responses to all four dengue virus serotypes after one or two administrations. We noted asymmetric responses with higher antibody titres to

serotype 2 while still inducing seroconversion to the other three serotypes. Our findings emphasise the acceptable tolerability and immunogenicity of the tetravalent DENVax formulations in healthy, dengue-naive adults. Phase 3 efficacy trials and regulatory approval would allow for implementation of these promising vaccines to prevent dengue fever in endemic countries and in travellers.

Discussion

The two different formulations (low and high dose) of the tetravalent DENVax vaccine were well tolerated after either subcutaneous or intradermal administration to flavivirus-naive adults. No clinically meaningful changes were shown in either clinical chemistry or haematology variables, or vaccine-related serious adverse events or withdrawals caused by adverse events. Fevers and rashes were uncommon and there was no indication of transient neutropenia or lymphopenia, which have been reported with other live, attenuated dengue vaccines.[17,18] We did not note any clustering of dengue-like symptoms, such as fever in conjunction with rash, retro-orbital pain, or muscle or joint pain. We noted frequent local reactogenicity, indicative of an inflammatory reaction at the injection site. The local reactions were mostly mild with more reactions recorded after intradermal administration compared with the subcutaneous route.

The short duration of DENVax RNA replication, the low levels of vaccine viraemia, and the genetic stability of the attenuation determinants in this trial also support the safety of DENVax. The levels of DENVax viraemia were very low and unlikely to lead to transmission to mosquitoes.[19] Furthermore, the attenuating mutations present in each of the DENVax constructs prevent replication and transmission of the vaccine viruses in mosquitoes.[14,20] No clinical signs or changes in clinical laboratory values were associated with viraemia or with reversion of one (5′ NCR-57 locus) of the three attenuating determinants in DENVax-2 or DENVax-4. These data are consistent with the molecular dissection of the attenuating mutations in the parent-attenuated seortype-2 virus; presence of mutations in NS1 and NS3 are sufficient to retain all attenuation phenotypes of DENVax-2 in vitro and in mice and mosquitoes.[9,14] The presence of several attenuating mutations provides additional assurance against reversion to wild-type, unattenuated viruses.

After the first administration, the DENVax-2 strain was the most common viral RNA or infectious virus detected in blood samples. This result suggests that the chimeric DENVax-1, DENVax-3, or DENVax-4 components might have lower replicative capacity, are less immunogenic than DENVax-2, or are subject to interference in vivo. Indeed, replication of DENVax-3 and DENVax-4 was rarely noted and low neutralising antibody titres were noted against these serotypes. Surprisingly, we did not record replication of DENVax-1 in this study or in previous preclinical studies, despite the high neutralising antibody titres to serotype 1.[12,13] The lower titres to serotypes 3 and 4 by the tetravalent formulations in this trial are consistent with our previous findings in preclinical studies; DENVax provided protection against all four dengue viruses in non-human primates, including serotype-4 challenge,[12] despite the lower antibody responses to that serotype.

In this study DENVax induced neutralising antibody responses to all four dengue viruses. After first administration, more than 80% of patients seroconverted to two or more dengue viruses in all four groups, and more than 80% of patients seroconverted to three or more virus serotypes after one subcutaneous or intradermal administration of the high-dose formulation. Independent of formulation and route of administration, after two administrations of DENVax all patients seroconverted to at least one serotype, more than 90% of patients seroconverted to two or more serotypes, more than 85% seroconverted to at least three serotypes, and more than 47% of patients seroconverted to all four serotypes. The second administration increased the seroconversion to multiple serotypes. Our results also show that either intradermal or subcutaneous routes of administration can induce tetravalent dengue responses. Subcutaneous administration might be favoured because it produced lower rates of local adverse events than did intradermal administration. Because subcutaneous administration is also more accepted in clinical practice, this route could aid implementation of mass vaccination campaigns.

The search for an effective dengue vaccine has been hampered by the scarcity of correlates of protection.[8] For many years, several assumptions have been made about protection against dengue virus: that neutralising antibodies are the main targets for protection, that balanced antibody responses to all four virus serotypes are needed, and that cellular immune responses might not be needed for protection. The results of a phase 2b efficacy trial suggest that these assumptions might need reassessment.[21] In that study, a tetravalent, recombinant, live, attenuated dengue vaccine based on the backbone of a yellow fever vaccine (17D) induced balanced titres of neutralising antibodies to all four dengue virus serotypes, yet did not protect against dengue fever caused by infection with serotype 2.[21] By contrast, in the present study, we recorded asymmetric responses with increased antibody titres to serotype 2, while still inducing seroconversion to the other three serotypes with a microneutralisation Vero-cell based $PRNT_{50}$ assay. In view of the low association between $PRNT_{50}$ titres and efficacy in the phase 2b clinical trial completed by Sanofi Pasteur,[8,21,22] we aim to explore the use of alternative assays to characterise the humoral and cellular immune response to DENVax in samples from this study and in future clinical trials.

Our phase 1 clinical trial supports the potential of DENVax as a vaccine against dengue (panel). Additional phase 1 and phase 2 clinical trials are underway. If effective, DENVax could greatly contribute to WHO's goal of reducing dengue mortality by 50% and morbidity by 25% by 2020.[51]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Sara Robledo, Juan Jose Fernandez, Jorge A Egurrola, Claudia Asela, and Eugenia Cardona from Programa de Estudio y Control de Enfermedades Tropicales (PECET) for their assistance in the study; the clinical laboratory Las Americas (Medellin); personnel from the Universidad de Antioquia, Carmen de Viboral for their participation in the study; Michele Hurliman and Matt Drietz for their assistance in undertaking the trial; and Keith Veitch, Taryn Rogalski, Anthony Edmonds, Derek Wallace, Wolfgang Bender, and Kwasi Amfo from Takeda Vaccines for the technical review of this manuscript.

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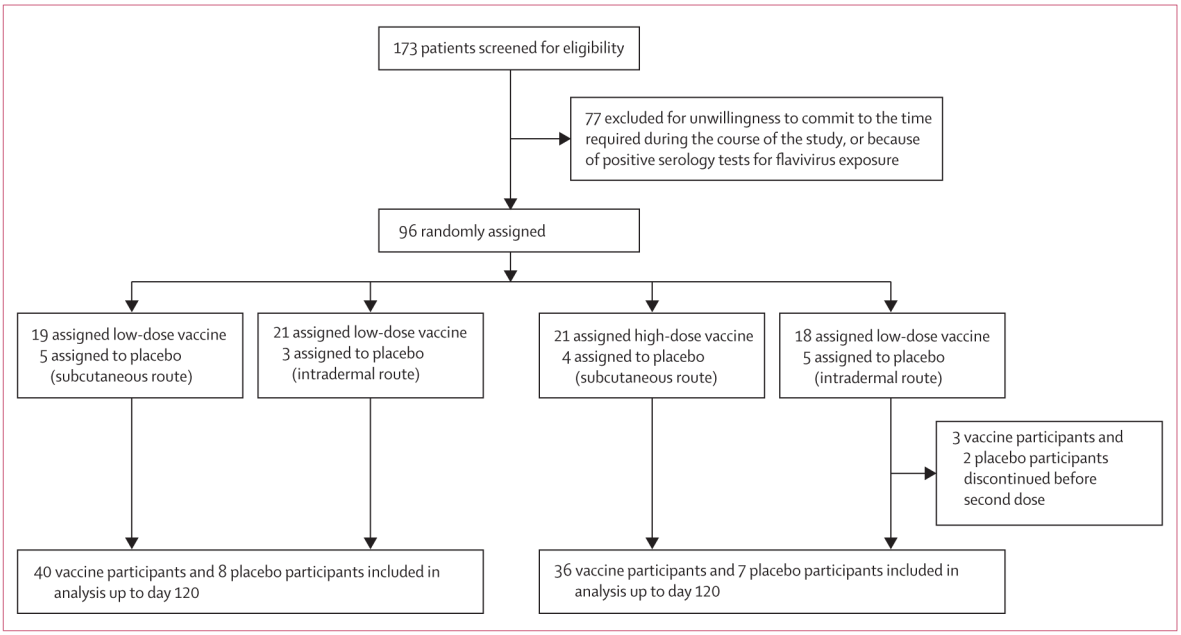

Figure 1.
Trial profile

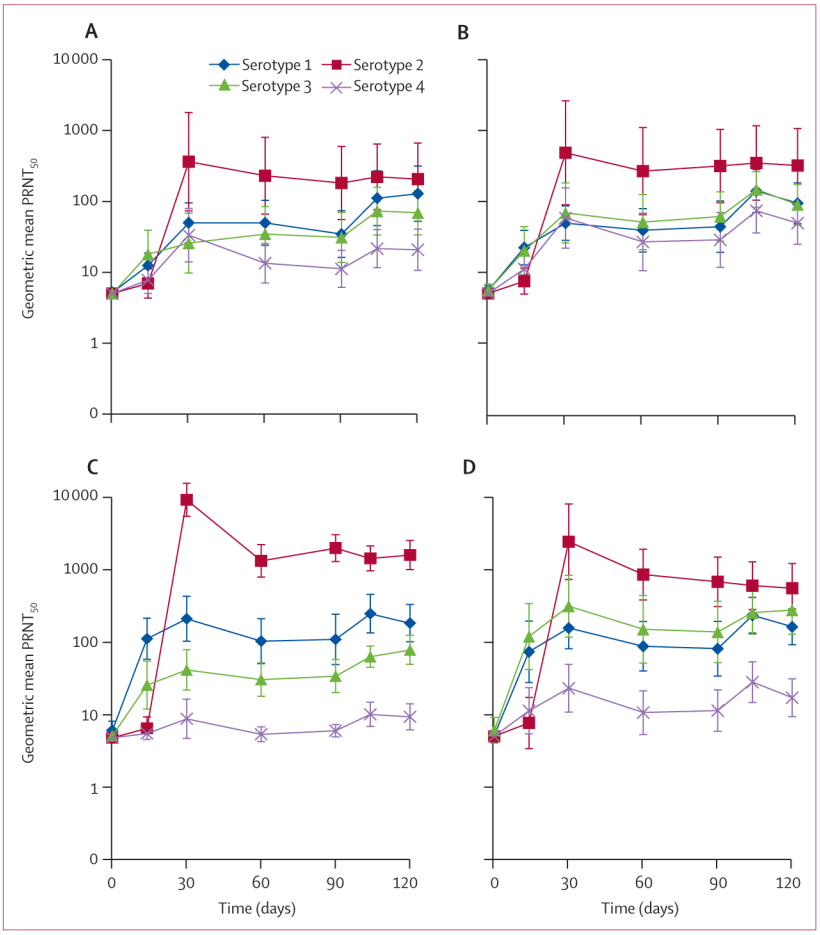

Figure 2. Neutralising antibody titre induced by DENVax formulations in study participants

Low dose, subcutaneous (A); low dose, intradermal (B); high dose, subcutaneous (C); high dose, intradermal (D). Error bars show 95% CIs around the geometric mean titres. PRNT=plaque-reduction neutralisation test.

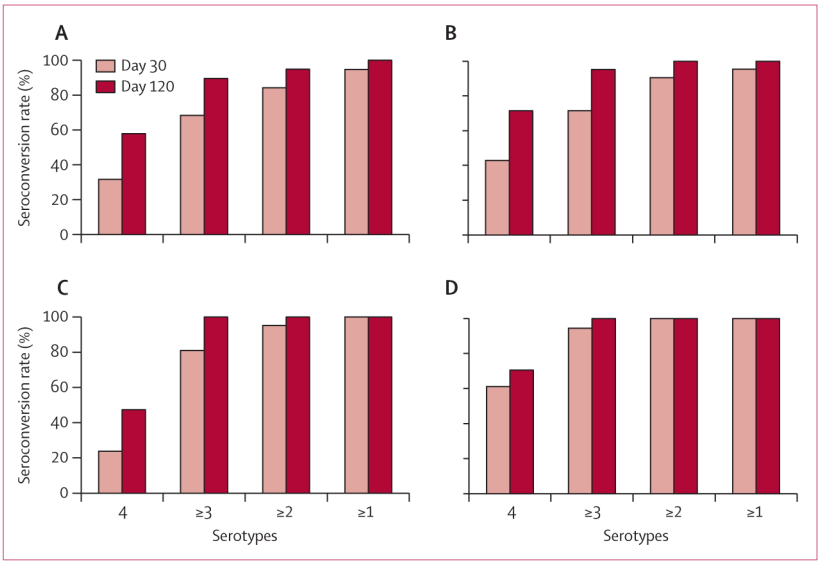

Figure 3. Seroconversion rates after dose one or dose two of DENVax

Low dose, subcutaneous (A); low dose, intradermal (B); high dose, subcutaneous (C); high dose, intradermal (D).

Table 1

Baseline characteristics

	Low-dose group		High-dose group	
	Subcutaneous (n=24)	**Intradermal (n=24)**	**Subcutaneous (n=25)**	**Intradermal (n=23)**
Sex				
Male	6 (25%)	11 (46%)	5 (20%)	7 (30%)
Female	18 (75%)	13 (54%)	20 (80%)	16 (70%)
Age at screening (years)	20·5 (18–29)	18·5 (18–33)	22·8 (18–45)	22·0 (18–42)
Weight (kg)	58·5 (47–83)	56·5 (40–85)	56·5 (44–78)	63·8 (44–90)
BMI (kg/m²)	21·4 (18·2–27·0)	20·7 (16·6–32·5)	22·5 (18·0–28·0)	23·1 (18·0–28·9)

Data are n (%) or median (range), unless otherwise indicated. BMI=body-mass index.

Table 2

Number of patients with at least one solicited or unsolicited adverse events after first or second dose combined

	Low-dose subcutaneous		Low-dose intradermal		High-dose subcutaneous		High-dose intradermal		Overall	
	DENVax (n=19)	Placebo (n=5)	DENVax (n=21)	Placebo (n=3)	DENVax (n=21)	Placebo (n=4)	DENVax (n=18)	Placebo (n=5)	DENVax (n=79)	Placebo (n=17)
Solicited adverse events	19 (100%)	4 (80%)	20 (95%)	3 (100%)	18 (86%)	4 (100%)	18 (100%)	4 (80%)	75 (95%)	15 (88%)
Local	15 (79%)	1 (20%)	20 (95%)	2 (67%)	14 (67%)	1 (2%)	18 (100%)	1 (20%)	67 (85%)	5 (29%)
Systemic	17 (90%)	4 (80%)	17 (81%)	2 (66%)	17 (81%)	3 (75%)	17 (94%)	4 (80%)	68 (86%)	13 (77%)
Unsolicited adverse events	18 (95%)	5 (100%)	16 (76%)	3 (100%)	16 (76%)	2 (50%)	15 (83%)	3 (60%)	65 (82%)	13 (77%)
Local	0	0	3 (14%)	0	1 (5%)	0	4 (22%)	0	8 (10%)	0
Systemic	18 (95%)	5 (100%)	16 (76%)	3 (100%)	16 (76%)	2 (50%)	14 (78%)	3 (60%)	64 (81%)	13 (77%)

Data are n (%), unless otherwise indicated.

Table 3

Vaccine viral RNA and infectious virus after the first administration

	DENVax-2		DENVax-3		DENVax-4	
	n*	Mean titre (SD)	n*	Mean titre (SD)	n*	Mean titre (SD)
Low dose						
Viral RNA†						
Day 7	3	3·9 (0·3)	1	4·0	1	4·0
Day 9	11	4·3 (0·3)	0	··	0	··
Day 11	11	4·2 (0·3)	0	··	0	··
Day 15	6	3·9 (0·5)	0	··	0	··
Infectious virus‡						
Day 7	0	··	0	··	1	430
Day 9	6	28 (20)	··	··	··	··
Day 11	3	10 (0)	··	··	··	··
Day 15	0	··	··	··	··	··
High dose						
Viral RNA†						
Day 5	2	3·8 (0·2)	1	4·1	0	··
Day 7	10	4·0 (0·3)	1	4·2 (0·1)	0	··
Day 9	14	4·3 (0·5)	1	4·0	0	··
Day 11	22	4·0 (0·3)	0	··	0	··
Day 14	18	4·2 (0·3)	0	··	0	··
Infectious virus‡						
Day 5	0	··	1	110	··	··
Day 7	2	10 (0)	1	20	··	··
Day 9	4	16·8 (13)	1	30	··	··
Day 11	2	10 (0)	··	··	··	··
Day 14	7	11·0 (4)	··	··	··	··

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Only days with positive samples are listed; days 2, 4, and 30 were negative for all viruses; no viral RNA were detected for DENVax-1.

*Number of patients testing positive on a given day.

†Titre for viral RNA expressed as log10 (genome equivalents per mL).

‡Titre for infectious virus expressed as plaque-forming units per mL.

Table 4

Rates of dengue virus seroconversion after DENVax administration

	Serotype 1	Serotype 2	Serotype 3	Serotype 4
Low dose				
Subcutaneous				
Day 30 after vaccination	89·5%	68·4%	57·9%	63·2%
Day 120 after vaccination	94·7%	78·9%	94·7%	73·7%
Intradermal				
Day 30 after vaccination	90·5%	66·7%	71·4%	71·4%
Day 120 after vaccination	100%	76·2%	95·2%	95·2%
High dose				
Subcutaneous				
Day 30 after vaccination	95·2%	100%	81%	23·8%
Day 120 after vaccination	100%	100%	100%	47·4%
Intradermal				
Day 30 after vaccination	100%	88·9%	94·4%	66·7%
Day 120 after vaccination	100%	94·1%	100%	76·5%