INFECTIOUS DISEASE

The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model

Beth D. Kirkpatrick,[1]* Stephen S. Whitehead,[2]* Kristen K. Pierce,[1] Cecilia M. Tibery,[3] Palmtama L. Grier,[3] Noreen A. Hynes,[4] Catherine J. Larsson,[1] Beulah P. Sabundayo,[3] Kawsar R. Talaat,[3] Anna Janiak,[3] Marya P. Carmolli,[1] Catherine J. Luke,[4] Sean A. Diehl,[1] Anna P. Durbin[3†]

A dengue human challenge model can be an important tool to identify candidate dengue vaccines that should be further evaluated in large efficacy trials in endemic areas. Dengue is responsible for about 390 million infections annually. Protective efficacy results for the most advanced dengue vaccine candidate (CYD) were disappointing despite its ability to induce neutralizing antibodies against all four dengue virus (DENV) serotypes. TV003 is a live attenuated tetravalent DENV vaccine currently in phase 2 evaluation. To better assess the protective efficacy of TV003, a randomized double-blind, placebo-controlled trial in which recipients of TV003 or placebo were challenged 6 months later with a DENV-2 strain, rDEN2Δ30, was conducted. The primary endpoint of the trial was protection against dengue infection, defined as rDEN2Δ30 viremia. Secondary endpoints were protection against rash and neutropenia. All 21 recipients of TV003 who were challenged with rDEN2Δ30 were protected from infection with rDEN2Δ30. None developed viremia, rash, or neutropenia after challenge. In contrast, 100% of the 20 placebo recipients who were challenged with rDEN2Δ30 developed viremia, 80% developed rash, and 20% developed neutropenia. TV003 induced complete protection against challenge with rDEN2Δ30 administered 6 months after vaccination. TV003 will be further evaluated in dengue-endemic areas. The controlled dengue human challenge model can accelerate vaccine development by evaluating the protection afforded by the vaccine, thereby eliminating poor candidates from further consideration before the initiation of large efficacy trials.

INTRODUCTION

Dengue viruses (DENVs) are the most prevalent mosquito-borne viruses in the world, with estimates of 390 million infections in more than 120 countries and more than 2 million cases of dengue hemorrhagic fever annually (*1*). Dengue infection ranges from an asymptomatic (most common) or mildly symptomatic illness to one that results in bleeding diatheses, plasma leakage, and vascular collapse (dengue hemorrhagic fever/shock syndrome). All four DENVs (DENV-1 to DENV-4) can cause the full spectrum of disease, and, although severe disease can occur after primary infection, epidemiologic studies have determined that preexisting immunity to one DENV serotype is the greatest risk factor for more severe disease upon secondary, heterotypic DENV infection (*2*–*4*). The association of a more severe dengue with a second, heterotypic DENV infection is thought to be mediated by the phenomenon of antibody-dependent enhancement of infection in which cross-reactive, non-neutralizing antibody is able to bind to the virus and allow entry of the virus-antibody complex through the FcγR (Fc-γ receptor) on monocytes and macrophages (*5*). Severe or enhanced disease can occur with first infection in infants because of the presence of passively transferred dengue maternal antibody (*2*, *6*). Maternal antibody initially provides protection against dengue, but as the antibody wanes, the once protective antibody enhances dengue infection in the infant. Because the risk of severe disease is greatest in hyperendemic areas, where multiple serotypes of DENV are circulating, and a vaccine that induces only partial protection may have long-term safety implications (*7*), an effective dengue vaccine must protect against all four serotypes (*8*).

There are several candidate dengue vaccines under clinical evaluation. The vaccine furthest along in development is CYD, a live attenuated tetravalent (LATV) chimeric three-dose vaccine in which the prM and E proteins of each DENV-1 to DENV-4 replace those of the yellow fever 17D virus (fig. S1). Three efficacy trials of CYD have been completed with varying results (*9*–*11*). Efficacy against symptomatic dengue ranged from 30.2% in a phase 2b study in Thailand (*9*) to 60.8% in a phase 3 study in Latin America (*11*). In the two trials conducted in Asia, the vaccine did not afford significant protection against symptomatic DENV-2 infection (*9*, *10*), despite evidence of seroconversion to DENV-2 in >95% of vaccine recipients. In both regions, the vaccine showed strong protection against hospitalization and severe dengue. In both phase 3 trials, the vaccine did not afford significant protection in subjects who were dengue-naïve before vaccination (*10*, *11*). Recent data from the long-term safety assessment demonstrated that in year 3 after vaccination, the risk of hospitalization was higher in CYD recipients compared with placebo recipients in subjects less than 9 years of age and that this risk was highest in subjects 2 to 5 years of age [relative risk (RR), 7.45] (*12*). The vaccine has recently been licensed in three countries, Mexico, the Philippines, and Brazil. Because of the lower efficacy in dengue-naïve individuals and the safety signal observed in children younger than 9 years, the vaccine was licensed for persons 9 to 45 years of age in the dengue-endemic areas of these countries. Neutralizing antibody appears to correlate with protection for other flavivirus vaccines such as YF17D and the live attenuated Japanese encephalitis vaccine SA-14-14-2 (*13*), and the antibody was previously thought to correlate for protection against dengue, but this was not evident with the antibody induced by CYD (*9*).

[1]Vaccine Testing Center, Department of Medicine, University of Vermont College of Medicine, Burlington, VT 05401, USA. [2]National Institutes of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. [3]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA. [4]Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.
*Co-first authors.
†Corresponding author. E-mail: adurbin1@jhu.edu

The lack of a known correlate of protection and the failure of a neutralizing antibody to correlate with protection against dengue has complicated dengue vaccine development (*14*). Not only can a dengue vaccine that induces only partial protection fail to prevent infection in some, but it may also actually increase the risk of severe dengue upon subsequent infection, as demonstrated in CYD14 (*12*). For this reason, dengue vaccine manufacturers require strong evidence of potential success of a vaccine before initiating large clinical trials in dengue-endemic areas. Without a correlate of protection, identifying these candidates may be difficult. For this reason, development of a controlled dengue human challenge model (DHCM) to better evaluate the candidate live attenuated dengue vaccine TV003 before its evaluation in dengue-endemic regions was sought.

The Laboratory of Infectious Diseases at the National Institutes of Health has evaluated numerous monovalent and tetravalent dengue candidate vaccines to identify candidates with the most acceptable safety, infectivity, and immunogenicity profile (*8*, *15*–*19*). TV003 is an admixture of four live attenuated recombinant dengue vaccine candidate viruses (rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30). It has been administered to 100 flavivirus-naïve healthy adults in three U.S. clinical trials (*8*, *19*). The vaccine induced a tetravalent antibody response in 74% of the subjects, with 92, 76, 97, and 100% of vaccinated subjects seroconverting to DENV-1, DENV-2, DENV-3, and DENV-4, respectively (*19*).

Before the initiation of large efficacy trials in endemic regions, it seemed prudent to assess the protective efficacy afforded by vaccination with TV003, particularly in those individuals seronegative to dengue before vaccination. Because the DENV-2 component of TV003 induced the lowest frequency of seroconversion and the efficacy of CYD was lowest against DENV-2, the ability of the vaccine to protect against DENV-2 infection was of greatest interest and a DENV-2 human challenge model was used. The challenge virus rDEN2Δ30 is derived from an American genotype DENV-2 that was isolated during an outbreak of dengue in the Kingdom of Tonga in 1974 (*20*). This outbreak was notable for causing mild disease and for a virus isolation rate that was considerably lower than that of other DENV-2 outbreaks (*20*). rDEN2Δ30 was originally developed as a DENV-2 candidate vaccine virus; however, it was not sufficiently attenuated in preclinical studies when compared with its parent virus (*21*). When evaluated in normal healthy volunteers at a dose of 10^3 plaque-forming units (PFU), it induced features characteristic of dengue infection: viremia in 100% of the subjects (mean peak titer of 2.5 $\log_{10}$ PFU/ml), rash in 80%, and neutropenia in 40% (*22*). For these reasons, rDEN2Δ30 was abandoned as a candidate vaccine virus and instead was chosen for use as a DENV-2 challenge virus. Here, the ability of a single dose of TV003 to protect against infection with the DENV-2 challenge virus rDEN2Δ30 administered 6 months after vaccination was evaluated.

RESULTS

Study participants

A total of 48 flavivirus-naïve subjects were enrolled (Fig. 1); 24 subjects were enrolled at each site. There were no statistically significant differences in the mean age (29.4 years versus 30.8 ± 1.7 years), gender (54.2% male versus 66.7% male), or race (58.3% White versus 62.5% White) in TV003 recipients compared to control recipients, respectively. Forty-one subjects returned for challenge with rDEN2Δ30 (21 TV003 recipients and 20 placebo recipients). There were no significant differences in the age, gender, or race between the two groups at challenge.

TV003 vaccination

Vaccination was well tolerated, and fever was not observed. The only adverse event (AE) that occurred more frequently in TV003 recipients compared to placebo controls was a mild, asymptomatic rash in 79.2% of vaccine recipients (table S1). The rash consisted of a few maculopapular lesions on the proximal upper extremities and chest. It was unnoticed by the subjects and resolved in 5 to 10 days. Vaccine virus was recovered from 17 (71%) of 24 TV003 recipients (Table 1). Multiple viruses were recovered from 8 of 17 TV003 recipients. The DENV-3 component was recovered most frequently (63%), followed by the DENV-1 component (21%), the DENV-4 component (17%), and the DENV-2 component (4%). After a single dose of TV003, 91.7% of subjects seroconverted to all four DENV serotypes (Table 2). All 24 TV003 recipients seroconverted to DENV-2, DENV-3, and DENV-4, whereas 91.7% seroconverted to DENV-1.

Challenge of vaccine recipients with rDEN2Δ30

After challenge, all subjects who had received TV003 were completely protected from infection with rDEN2Δ30. Challenge virus was not recovered from any of the 21 subjects who received TV003 at study day 0 and were challenged ($P < 0.0001$), nor did any develop rash ($P < 0.0001$), neutropenia ($P = 0.048$), or thrombocytopenia (Table 3). Thus, TV003 induced complete protection against the protocol-defined endpoints of viremia, rash, and neutropenia induced by rDEN2Δ30. In TV003 recipients, the geometric mean $PRNT_{50}$ (GMT) to DENV-2 was less than fourfold from the study day 180 prechallenge GMT of 1:49.6 to the peak postchallenge GMT of 1:123.5 (Table 4). This titer was significantly lower than the peak GMT achieved in controls after challenge (1:517; $P = 0.0012$). Of the 21 TV003 recipients who received the challenge virus, 12 had less than a 4-fold rise in neutralizing antibody titer (sterilizing immunity) and 9 had a ≥4-fold rise (nonsterilizing immunity). Of interest, the prechallenge GMT in subjects who exhibited sterilizing immunity was significantly higher (1:90) compared to those who did not exhibit sterilizing immunity (1:35; $P = 0.03$), suggesting that neutralizing antibody may be an important component of protection against DENV infection.

In sharp contrast to subjects vaccinated with TV003, rDEN2Δ30 was recovered from the blood of all 20 placebo recipients who were challenged (Table 3) (mean peak titer, 2.3 ± 0.1 $\log_{10}$ PFU/ml; maximum titer, 2.9 $\log_{10}$ PFU/ml; mean day of onset, 4.7; mean duration of viremia, 6.1 days). Nineteen (95%) had virus recovered on multiple days. A more expansive maculopapular rash was observed in 16 of 20 (80%) control subjects after challenge and was graded as moderate severity in 6 of 16 (38%). In addition, four control subjects (20%) developed a transient neutropenia after challenge. Neutropenia was graded as moderate [absolute neutrophil count (ANC), 500 to 749/mm^3] in three. Two control subjects (10%) developed thrombocytopenia (platelet count, <100,000/mm^3).

DISCUSSION

The single-dose, LATV dengue vaccine TV003 induced complete protection against all primary and secondary endpoints of infection after challenge with the DENV-2 strain rDEN2Δ30 administered 6 months

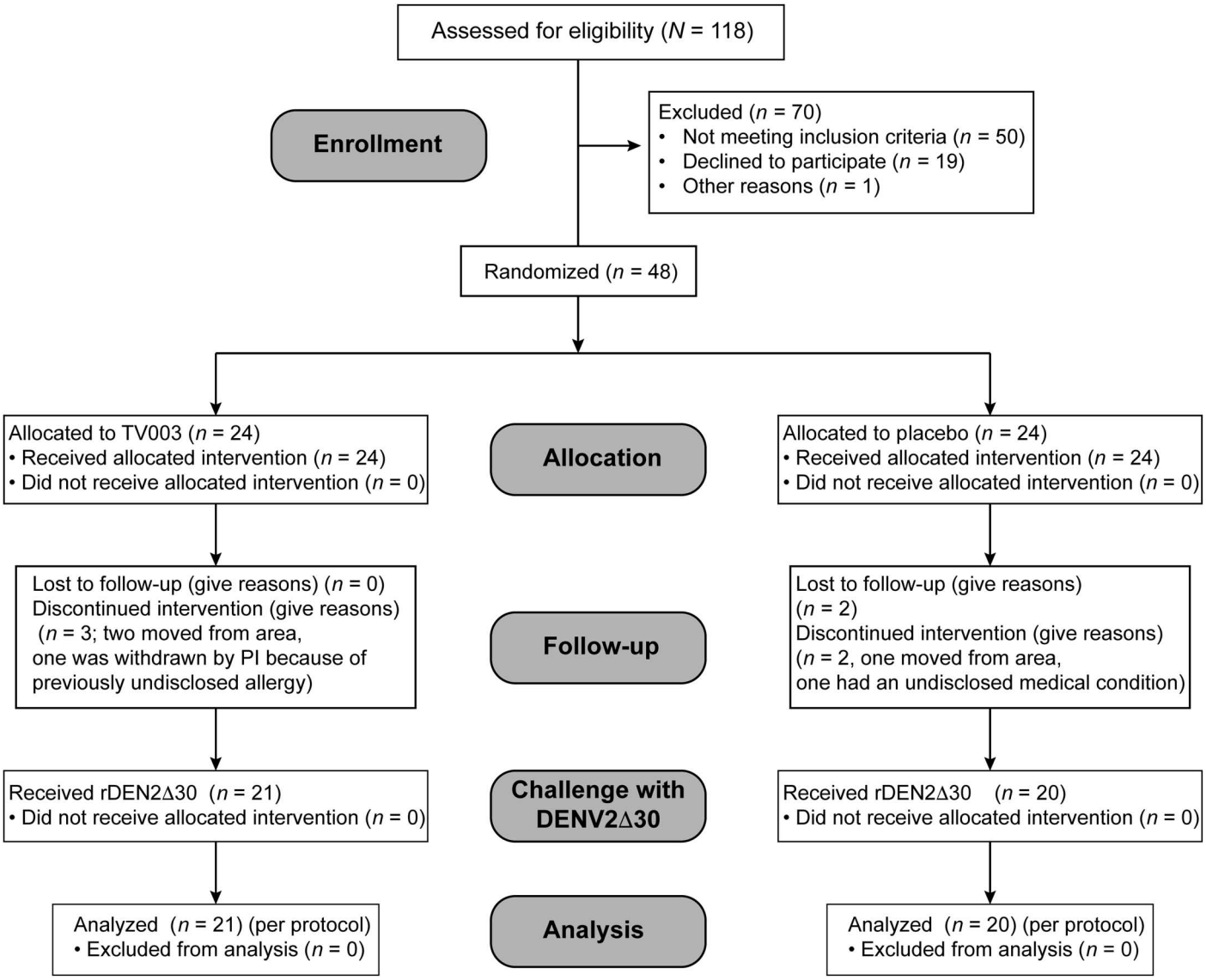

Fig. 1. Screening, enrollment, and follow-up of flavivirus-seronegative subjects for vaccination with the dengue vaccine TV003 and challenge with DENV-2 6 months later. As described in the text, 48 subjects were randomly assigned to receive TV003 or placebo on study day 0. The treatment assignment was masked to study staff and volunteers. On study day 180, all eligible subjects (41) received the challenge virus rDEN2Δ30. PI, principal investigator.

after vaccination. Vaccinated subjects did not experience viremia, rash, or neutropenia after challenge. Furthermore, 12 of 21 vaccinated subjects demonstrated sterilizing immunity to challenge with rDEN2Δ30 as evidenced by undetectable viremia and an inability to further boost antibody titers (<4-fold rise in $PRNT_{50}$ against DENV-2; Table 4). The remaining nine subjects did not have detectable viremia but did have a ≥4-fold rise in $PRNT_{50}$ to DENV-2, indicating there may have been subtle viral replication below the level of detection that was sufficient to boost the antibody response while still protecting from clinically apparent infection.

This work is important for the rapidly changing landscape of dengue vaccine development. The low efficacy of the multidose CYD against DENV-2, particularly in DENV-naïve individuals and despite its ability to induce neutralizing antibody, has complicated the clinical development of dengue vaccines. The decision to embark on efficacy trials in endemic regions must be made with the knowledge that a poorly effective vaccine may, in the long run, pose a greater risk for developing severe dengue than no vaccine, possibly due to immune enhancement of disease. This has become evident in the phase 3 trial of CYD, wherein children who were 2 to 5 years of age at the time of vaccination with CYD had an RR of 7.45 for hospitalization in year 3 of the trial (2 years after the last vaccination) due to dengue compared to placebo recipients (*12*). For this reason, a safe DHCM as a means to evaluate the protective efficacy of TV003 live attenuated dengue vaccine against DENV-2 before initiating efficacy trials in endemic areas was developed.

The goal of the study was to design a model that could reproducibly produce objective clinical and virologic outcomes with a sufficient frequency to power studies for significance with relatively few numbers

Table 1. Summary of viremia after administration of TV003 (CIR287, *n* = 24).

Admixture	Vaccine components	% with viremia	Mean peak titer ± SE (log_{10} PFU/ml)*	Maximum titer (log_{10} PFU/ml)	Mean day of onset (range)	Mean duration in days (range)
TV003	DEN1Δ30	21	0.7 ± 0.2	1.7	7.6 (2–10)	5.0 (2–7)
	DEN2/4Δ30	4	0.5 ± 0.0	0.5	8.0 (all 8)	1.0 (all 1)
	DEN3-3Δ30/31-7164	63	0.6 ± 0.1	1.3	8.6 (6–14)	3.5 (1–7)
	DEN4Δ30	17	0.6 ± 0.1	1.0	8.0 (6–10)	1.3 (1–2)
	Total %	**71**				

Lower limit of detection = 0.5 log_{10} PFU/ml.

Table 2. Serologic response of flavivirus-naïve subjects after vaccination with TV003.

	Peak geometric mean titer, reciprocal (median)*			
n	DEN1	DEN2	DEN3	DEN4
24	47 (29)	84 (78)	152 (167)	270 (276)
Range	<5–499	1–710	31–533	43–5025
	% with $PRNT_{50}$ ≥1:10			
n	DEN1	DEN2	DEN3	DEN4
24	91.7	100	100	100
	Multivalent responses (cumulative)			
n	Tetra	Tri	Bi	Mono
24	91.7%	8.3% (100)	0	0

*Titer calculated only for those subjects who had a $PRNT_{50}$ ≥10. The peak titer is calculated from titers obtained through study 90 after vaccination (days 28, 56, and 90).

of subjects. In addition, a model that induced the same type of clinical infection outcome that occurs most frequently in natural dengue infection was preferred. Mild dengue infection without fever is not uncommon and may be important in transmission of the virus (*23*–*25*). The estimated majority (~75%) of natural dengue infections do not present for clinical care; they are asymptomatic or only mildly symptomatic (*1*). Thus, it seemed unreasonable to develop a model that induced dengue fever or severe dengue in volunteers because inducing a symptomatic febrile illness in a sufficient proportion of subjects to power studies for significance could pose an unacceptable safety risk to some subjects who may develop severe or very serious disease. The challenge model described here is similar to that of the controlled human challenge model for malaria in which infection, not disease, is the chosen endpoint (*26*). This differs somewhat from the model described by Sun *et al.* in which the clinical endpoint of challenge was the induction of dengue disease and required subjects to be housed for clinical observation for study days 4 to 17 (*27*). In this case, DENV-1 and DENV-3, both failed candidate dengue vaccines, were used as challenge viruses. There were only two control subjects who received the DENV-1 challenge virus and two who received the DENV-3 challenge virus. For this reason, the study was not powered to detect a significant difference in the outcomes between those who had previously received LATV dengue vaccine and controls. Illness was graded on the basis of objective and subjective clinical findings (a reactogenicity index). Although fever was described in both control subjects of the DENV-1 challenge, the challenge virus was recovered from only one of two control subjects (50%), questioning whether the fever and symptoms described were due to dengue or another intercurrent viral infection. In addition, the clinical symptoms described in both DENV-1 control subjects developed quite late (≥19 days after infection), which is unusual for dengue. Here, viremia and rash were observed in most (80%) of the control subjects. The use of dengue infection (as defined primarily by viremia) as opposed to dengue disease as the endpoint of a DHCM minimizes the risk to subjects while still enabling a relevant test of vaccine efficacy.

A minor limitation of the current study is the challenge time point of 6 months after vaccination. Challenge at the 6-month time point was chosen for the following reasons. DENVs are thought to induce lifelong homotypic protection against disease but only short-term heterotypic protection of about 2 to 3 months. A. Sabin demonstrated that protection against infection with a heterologous DENV lasted about 2 months (*28*). If a heterologous DENV was administered 3 to 9 months after primary infection (the longest duration evaluated), the illness induced by the heterologous virus was modified but virus was recovered, indicating that heterologous infection can occur as early as 3 months after the primary infection (*28*). Others have noted that immunity induced by the first DENV infection can protect against symptomatic febrile illness after the second DENV infection for almost 2 years (*29*). In studies of CYD in flavivirus-naïve subjects, the DENV-4 vaccine component was recovered in several subjects after the second dose given at 6 months (*30*, *31*), indicating that the vaccine was unable to induce sterilizing immunity against the attenuated vaccine virus for 6 months. Although protection against viremia was

Table 3. Clinical response to rDEN2Δ30 in subjects who had received TV003 or control 6 months earlier. ALT, alanine transaminase; PT, prothrombin time; PTT, partial thromboplastin time.

AE	Primary treatment	
	TV003 (n = 21)	Placebo (n = 20)
Injection site		
Erythema	9.5%	0.0%
Pain	0.0%	0.0%
Tenderness	0.0%	0.0%
Induration	0.0%	0.0%
Systemic		
Viremia	0.0%	100.0%*
Fever	0.0%	0.0%
Headache	23.8%	30.0%
Rash	0.0%	80.0%*
Neutropenia	0.0%	20.0%†
Elevated ALT	0.0%	5.0%
Myalgia	4.8%	20.0%
Arthralgia	0.0%	10.0%
Retro-orbital pain	9.5%	25.0%
Fatigue	14.3%	15.0%
Nausea	14.3%	20.0%
Photophobia	4.8%	0.0%
Elevated PT	0.0%	0.0%
Elevated PTT	4.8%	0.0%
Thrombocytopenia	0.0%	10.0%

*P < 0.0001, two-sided. †P = 0.048, two-sided.

Table 4. Most of the TV003 recipients demonstrate no boost to DENV-2 after challenge.

Subject	$PRNT_{50}$ day 180	Peak $PRNT_{50}$ post-challenge	Ratio $PRNT_{50}$ post/pre-challenge*
1	49.7	19.1	0.4
2	72.0	48.1	0.7
3	50.6	38.3	0.8
4	66.5	56.6	0.9
5	55.6	49.6	0.9
6	75.3	78.4	1.0
7	260.2	324.5	1.2
8	119.9	160.9	1.3
9	196.5	301.2	1.5
10	14.8	23.1	1.6
11	58.2	97.1	1.7
12	55.9	145.4	2.6
13	**12.3**	**49.3**	**4.0**
14	**51.9**	**209.9**	**4.0**
15	**19.6**	**80.8**	**4.1**
16	**45.2**	**227.5**	**5.0**
17	**27.3**	**139.6**	**5.1**
18	**40.3**	**251.8**	**6.2**
19	**46.0**	**637.1**	**13.9**
20	**19.3**	**373.6**	**19.4**
21	**48.8**	**1336.1**	**27.4**
Geomean	49.6	123.5	2.5

*Samples with a post/pre-$PRNT_{50}$ ratio ≥4.0 are bolded.

induced by a second dose of TV003 given at 6 months (*19*), a more rigorous challenge with rDEN2Δ30 was sought to better assess the protection afforded by TV003. In addition, to maximize the retention of subjects for challenge, a 6-month interval between vaccination and challenge was chosen. Given the high level of protection afforded by TV003 against challenge with rDEN2Δ30 at 6 months and the possible role of heterotypic protection afforded by DENV-1, DENV-3, and DENV-4 vaccine components, additional studies to strategically push the limits of protection with the goal of identifying correlate(s) of protection are under consideration. Evaluation of the ability of a formulation consisting of the DENV-1, DENV-3, and DENV-4 components of TV003 to protect against rDEN2Δ30 (NCT02433652) is currently ongoing.

There are several differences in the composition of TV003 and the immune response it induces that distinguish this vaccine from CYD and may contribute to the high level of protection observed for TV003 against rDEN2Δ30. First, three of the four DENV serotypes contained within TV003 are full-length viruses that contain all wild-type structural and nonstructural proteins; the fourth virus (DEN2/4Δ30) is a chimeric virus. Because most of the $CD8^+$ T cell epitopes are contained in the nonstructural proteins (*32*), TV003 induces a strong cellular immune response to all DENVs (*33*). As described by Weiskopf *et al.*, when the four components of TV003 are administered as monovalent viruses, they are capable of inducing serotype-specific cell-mediated immune responses. However, when they are administered as a tetravalent admixture (TV003), the CD8 T cell response converges to recognize epitopes that are highly conserved among all four DENV serotypes (*33*). Second, each of the four components of TV003 is highly infectious; a single dose of TV003 induces viremia with all four serotypes and seroconversion rates of 76% to DENV-2, 92% to DENV-1, 95% to DENV-3, and 100% to DENV-4 in flavivirus-naïve adults (*19*). A single dose of CYD in flavivirus-naïve subjects, however, induced seroconversion to DENV-1 in fewer than 18% of the subjects and to DENV-2 in fewer than 40% (*34*). In addition, antibody titers were significantly boosted to DENV-1 and to DENV-2 with the second and third doses of CYD, indicating that multiple doses of the vaccine were unable to induce sterilizing immunity. The poorer infectivity of the DENV-1 and DENV-2 components of CYD and its inability to induce a strong cellular immune response to DENV may contribute to its weaker efficacy against DENV-2 and DENV-1.

The strong protective immunity against DENV-2 induced by TV003 is likely a combination of both a strong homotypic antibody response against DENV-2 that can prevent infection in most of the subjects and a cellular immune response that controls infection if it does occur. Under investigation are the roles of both in the protective immune response to

dengue to better define a correlate of vaccine efficacy. Of particular interest is determining the importance of differences in the immune response of those who demonstrated sterilizing immunity to challenge and those who did not. As discussed earlier, those who developed sterilizing immunity against challenge had a significantly higher neutralizing antibody titer to DENV-2 at the time of challenge than those who did not. Assays are currently under development to further explore the polyclonal antibody response to TV003 in more detail, including mapping the antibody repertoire induced by rDEN2Δ30 and TV003.

In the absence of a proven correlate of protection for dengue, the DHCM offers insight in the early assessment of efficacy for TV003 and other candidate DENV vaccines as well as a tool to explore putative correlate(s) of protection. Because rDEN2Δ30 induces viremia in 100% of flavivirus-naïve subjects, challenge studies can be powered for efficacy in a relatively small number of subjects. In addition, because the clinical signs associated with rDEN2Δ30 infection are mild or moderate rash and neutropenia, studies can be conducted on an outpatient basis without the need for intensive clinical monitoring and management.

In conclusion, a DHCM can be a useful tool to downselect candidate dengue vaccines before the initiation of large efficacy trials in endemic areas. Although human challenge models can be useful to identify promising (or poor) candidate vaccines, they are not a substitute of efficacy trials. Although TV003 induced complete protection against the DENV-2 challenge virus given 6 months after vaccination, the true efficacy of TV003 can only be established by performing a phase 3 trial in endemic areas. In addition, the long-term protection afforded by TV003 can only be assessed from phase 3 efficacy trials that include a long-term follow-up and from postlicensure studies, should the vaccine be licensed in endemic areas. As such, a phase 3 trial of TV003, produced by the Instituto Butantan in São Paulo, Brazil, is scheduled to begin in early 2016. This phase 3 trial will provide the opportunity to validate any putative correlate(s) of protection identified by the DHCM. Once validated, such a correlate can be used to better understand dengue disease as well as to accelerate vaccine development.

MATERIALS AND METHODS

Study oversight

The studies were performed under an investigational new drug application reviewed by the U.S. Food and Drug Administration and approved by the Institutional Review Boards at the University of Vermont and Johns Hopkins University. Informed consent was obtained in accordance with federal and international regulations (21CFR50 and ICHE6). External independent monitoring was performed, and the National Institute of Allergy and Infectious Diseases Data Safety Monitoring Board reviewed all safety data every 6 months.

Study design

This randomized, double-blind, placebo-controlled trial was conducted in Baltimore, MD, and Burlington, VT, and was designed to assess the protective efficacy of TV003 against DENV-2. Subjects were enrolled between 11 November 2013 and 25 February 2014 under study protocol CIR287 (ClinicalTrials.gov NCT02021968). A total of 48 subjects were enrolled in the trial. Twenty-four subjects were enrolled at each site (12 TV003 recipients and 12 placebo recipients). The dates of vaccination with TV003 and challenge with rDEN2Δ30 are provided in table S2. The primary efficacy endpoint of the study was the protection afforded by the vaccine against viremia induced by the challenge virus rDEN2Δ30. The study was powered to detect a protective efficacy against viremia of 60% at $\alpha = 0.05$ with 80% power. Secondary endpoints included protection against rash and neutropenia. At study day 0, 24 randomized subjects received a single subcutaneous dose of TV003, and 24 randomized subjects received a placebo control (vaccine diluent). Six months later, all subjects received 10^3 PFU of the DENV-2 challenge virus rDEN2Δ30 by subcutaneous injection.

Vaccine (TV003) and challenge virus (rDEN2Δ30)

TV003 is an admixture composed of three DENVs attenuated by deletion(s) in the 3′ untranslated region (3′UTR): rDEN1Δ30, rDEN3Δ30/31, and rDEN4Δ30, and a fourth component that is a chimeric virus with the prM and E proteins of DENV-2 NGC (New Guinea C strain) exchanged for DENV-4 in the rDEN4Δ30 genome (rDEN2/4Δ30) (fig. S1). The most common AE after TV003 administration is a mild, asymptomatic rash that correlates with seroconversion to all four DENV serotypes (*8*, *19*). The challenge strain rDEN2Δ30 is a recombinant virus derived from the DENV-2 Tonga/74 wild-type virus, a different genotype than DEN2 NGC.

Study assessment

On study day 0, subjects were administered 10^3 PFU of TV003 ($n = 24$) or control (L-15 vaccine diluent, $n = 24$); both were subcutaneously injected at a dose of 0.5 ml. Subjects were followed as outpatients, and their oral temperature was recorded three times daily for 16 days. Clinical assessments and physical examinations were performed every other day through study day 16 and on study days 21, 28, 56, 150, and 180 as described elsewhere (*8*). At study day 180, all subjects were challenged with 10^3 PFU of rDEN2Δ30 administered by subcutaneous injection. The schedule of study visits after challenge was the same as the schedule after the first visit with the exception of the study day 150 visit, which was not performed.

Adverse events

AEs and clinical laboratory results were captured, recorded, and graded for intensity and relationship to vaccination using protocol-defined grading and standard toxicity tables as previously described (*8*). Infection with TV003 or rDEN2Δ30 was defined as recovery of virus from the blood and/or seroconversion to DENV, as measured by the plaque reduction neutralization assay (PRNT). Sterilizing immunity induced by TV003 was defined as the prevention of rDEN2Δ30 infection as evidenced by a lack of viremia, rash, neutropenia, or antibody boost (≥4-fold rise in antibody titer) after challenge.

Virus quantitation and serologic response

Serum samples collected every other day at follow-up visits through day 16 were tested for viable virus by amplification and direct titration on Vero cell monolayers using serotype-specific monoclonal antibodies as described (*8*, *35*). Neutralizing antibody response was measured by PRNT assay, using the lowest serum dilution that gave a 50% reduction in viral foci ($PRNT_{50}$) in accordance with other live attenuated DENV vaccine evaluations (*8*, *35*). $PRNT_{50}$ assays used an initial serum dilution of 1:5. Seroconversion after first dose was defined as a $PRNT_{50}$ of ≥1:10 by study day 90 (fourfold rise in titer of 1:2.5 assigned to samples with undetectable titers <1:5). A boost in neutralizing antibody after rDEN2Δ30 challenge was defined as a ≥4-fold rise in serum neutralizing antibody titer by study day 270 compared with study day 180.

Data analysis

A per-protocol analysis was performed. χ^2 analysis with likelihood ratio determined statistically significant differences in the frequency of AEs or demographic characteristics and determined significant differences in the percent viremic, seroconverted, and tetravalent responders between TV003 and control groups after dose 1 and then after challenge. A Bonferroni correction was made for multiple comparisons. Paired *t* tests determined differences between peak serologic responses to DENV-2 after challenge. Statistical analysis was performed using JMP (version 9.0.2, SAS Institute Inc., 1989–2007) software.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/8/330/330ra36/DC1
Fig. S1. Components of the LATV dengue vaccines TV003 and CYD (Dengvaxia).
Table S1. Incidence of specific AEs in TV003 recipients compared to controls.
Table S2. Interval between TV003 and challenge.
Other supplemental material S1. Clinical protocol for the study "A Phase 1 Evaluation of the Protective Efficacy of a Single Dose of the Live Attenuated Tetravalent Dengue Vaccine TV003 to Protect Against Infection with Attenuated DENV-2, rDEN2Δ30-7169."
Other supplemental material S1. Consent form for the study "A Phase 1 Evaluation of the Protective Efficacy of a Single Dose of the Live Attenuated Tetravalent Dengue Vaccine TV003 to Protect Against Infection with Attenuated DENV-2, rDEN2Δ30-7169."
Other supplemental material S2. Excel file of screening and enrollment data.

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Acknowledgments: We thank all of the dedicated volunteers as well as the support of the University of Vermont (UVM) Medical Center Clinical Research Center and the Johns Hopkins University (JHU) Center for Immunization Research and their excellent research nurses and staff. We would also like to thank the Office of Clinical Research Policy and Regulatory Operations at the NIH for providing the regulatory oversight of this trial. **Funding:** This work was supported by the National Institutes of Allergy and Infectious Diseases Intramural Research Program, NIH (contract no. HHSN272200900010C). **Author contributions:** A.P.D., B.D.K., and S.S.W. prepared the manuscript. A.P.D., B.D.K., C. J. Luke, and S.S.W. designed the clinical trial. A.P.D., B.D.K., K.K.P., C.M.T., P.L.G., N.A.H., C. J. Larsson, K.R.T., and B.P.S. conducted the clinical trial. M.P.C., A.J., and S.A.D. performed the laboratory analysis. A.P.D. and S.S.W. conducted the data and statistical analysis. **Competing interests:** S.S.W. is an inventor on patent 8,039,003 "Recombinant attenuated dengue viruses comprising a deletion in the 3′untranslated region and additional attenuating mutations induced by chemical mutagenesis," patent 8,075,903 "Dengue tetravalent vaccine containing a common 30 nucleoside deletion in the 3′UTR of dengue types 1, 2, 3, and 4 or antigenic chimeric dengue viruses 1, 2, 3, and 4," and patent 8,337,860 "Development of dengue virus vaccine components." The other authors declare that they have no competing interests.

Submitted 26 December 2015
Accepted 5 February 2016
Published 16 March 2016
10.1126/scitranslmed.aaf1517

Citation: B. D. Kirkpatrick, S. S. Whitehead, K. K. Pierce, C. M. Tibery, P. L. Grier, N. A. Hynes, C. J. Larsson, B. P. Sabundayo, K. R. Talaat, A. Janiak, M. P. Carmolli, C. J. Luke, S. A. Diehl, A. P. Durbin, The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. *Sci. Transl. Med.* **8**, 330ra36 (2016).

Science Translational Medicine

The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model

Beth D. Kirkpatrick, Stephen S. Whitehead, Kristen K. Pierce, Cecilia M. Tibery, Palmtama L. Grier, Noreen A. Hynes, Catherine J. Larsson, Beulah P. Sabundayo, Kawsar R. Talaat, Anna Janiak, Marya P. Carmolli, Catherine J. Luke, Sean A. Diehl and Anna P. Durbin

Sci Transl Med **8**, 330ra36330ra36.
DOI: 10.1126/scitranslmed.aaf1517

Dengue model rises to the challenge

Human efficacy testing remains a major hurdle in bringing new vaccine candidates to the clinic. In the absence of accepted correlates of protection, rounds of safety trials must be performed before efficacy can be tested in a large population in an endemic area. Kirkpatrick *et al*. have developed a controlled dengue human challenge model to assess the protective efficacy of the most clinically advanced dengue vaccine candidate. They found that TV003, a live attenuated dengue vaccine that induces antibodies to all four dengue virus serotypes, protected against infection of an attenuated virus in 21 recipients when compared with 20 nonvaccinated controls. This model may serve as an early check for dengue vaccine candidates, limiting the risk of conducting large unsuccessful trials.

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