Historical discourse on the development of the live attenuated tetravalent dengue vaccine candidate TV003/TV005

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Abstract

Dengue is the most important arboviral disease world-wide with an estimated 400 million annual infections. Dengvaxia™ is a live attenuated tetravalent vaccine recently licensed for dengue seropositive individuals aged 9 – 45 years. There is great need for a dengue vaccine that be given to dengue-naïve individuals and very young children. To that end, the U.S. NIH developed a live attenuated tetravalent dengue vaccine using an iterative approach evaluating the safety, infectivity, and immunogenicity of different candidates. This approach identified poor candidates which were then discarded from further evaluation. Each of the components of the tetravalent vaccine formulation is able to replicate to very low titer, inducing a homotypic immune response to each. The immune response elicited by the tetravalent vaccine is balanced, without immunodominance of one component. The vaccine was licensed by several manufacturers for development, including the Instituto Butantan which initiated a Phase 3 efficacy trial.

Keywords

dengue; Vaccine; human challenge; efficacy

Introduction

Dengue is the world's greatest arboviral threat with nearly 4 billion people at risk for infection in 141 countries and has become an increasing problem for travelers [1,2]. The incidence of dengue has increased substantially with estimates suggesting the number of symptomatic cases has doubled every decade between 1990 and 2013 [3]. Bhatt et al estimated 96 million apparent dengue infections occurred worldwide in 2010 in addition to an additional nearly 300 million inapparent infections [4]. A 2013 analysis estimated nearly 60 million symptomatic dengue infections at a cost of almost 9 billion USD [2]. More than

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10 million of these infections were treated in the hospital setting, often overwhelming the health care facilities. The global distribution of dengue and magnitude of international travel has made dengue an important threat to travelers as well [5,6].

Dengue is caused by four antigenically distinct viruses; DENV-1, DENV-2, DENV-3, and DENV-4. Each of these viruses can cause the full spectrum of dengue infection: asymptomatic infection or undifferentiated febrile illness (most common), uncomplicated dengue fever, or severe dengue comprised of vascular leak syndrome leading to shock. Although a prognostic indicator for progression to severe dengue has not been identified [7,8], epidemiologic studies have demonstrated that pre-existing immunity to one DENV serotype is the greatest risk factor for severe dengue upon secondary infection with a heterotypic DENV [9]. Cross-reactive antibody induced by the primary DENV infection can bind to heterotypic virus of the secondary infection but, instead of neutralizing the virus, the antibody-virus complex enters monocytes and macrophages via the Fcγ receptor; a mechanism termed antibody-dependent enhancement (ADE) of infection [10]. This functional increase in infection of target cells leads to higher viral titers which have been associated with more severe dengue disease [11]. For this reason, partial immunity to dengue is a risk factor for severe disease. Interestingly, symptomatic and severe dengue is rarely observed with third or fourth dengue infection [12,13]. Second, heterotypic dengue infection is thought to broaden both the humoral and cellular immune response resulting in a more protective immune response against third and fourth infections. Indeed, Dejnirattisai et al isolated monoclonal antibodies from plasmablasts collected following secondary DENV infection which were broadly cross-reactive and highly neutralizing [14]. Additionally, Weiskopf et al demonstrated that secondary DENV infection honed CD8 T cell responses to conserved epitopes and that CD8+ T cell responses that were polyfunctional and of higher magnitude were associated with protection from dengue disease [15].

Although there is not an approved antiviral agent for the treatment of dengue, there is one licensed vaccine for the prevention of dengue and two other candidate dengue vaccines in Phase 3 clinical trial. Dengvaxia™, a live attenuated tetravalent vaccine, is the first dengue vaccine to be licensed. This vaccine is comprised of 4 chimeric viruses in which the prM and E proteins of YF17D are replaced with those of DENV-1, −2, −3, or −4. Because the non-structural proteins are those of YF17D and not of DENV, and because > 80% of the CD8 epitopes of DENV are found in the NS proteins [15], the CD8 T cell responses are directed primarily against YF and not dengue. Although the vaccine induced an overall efficacy of 60.3% against symptomatic dengue in the first 25 months, efficacy varied by serotype, age, and serostatus at vaccination [16–18]. Additionally, children who were 2 – 5 at the time of vaccination with Dengvaxia™, had a > 7-fold higher relative risk of hospitalization for dengue in year 3 of the study compared with those children who had received the control. Subsequent analysis linked the risk of more severe disease in the vaccinated group to being sero-naive to dengue at the time of vaccination [19,20]. For this reason, a pre-vaccination screening strategy was recommended for Dengvaxia™ [20,21]. Unfortunately, development of a screening assay with sufficient sensitivity and specificity to adequately determine serostatus prior to vaccination with Dengvaxia™ has been difficult [22,23].

The difference in efficacy by serotype and the risk of more severe disease in subjects who were dengue sero-naive at the time of vaccination is thought to be due an imbalance in the immune response to the vaccine [24]. The DENV-4 component of the vaccine is immunodominant; the neutralizing antibody titers to DENV-4 are significantly higher than the other serotypes following the first dose of vaccine in sero-naive subjects and additional doses do not boost DENV-4 titers but do boost titers to DENV-1, DENV-3, and DENV-3 [25,26]. Following vaccination of dengue sero-naive subjects, type-specific (homotypic) antibodies dominated the neutralizing antibody response to DENV-4 but more than 50% of the neutralizing antibody response to DENV-1, DENV-2, and DENV-3 was cross-reactive [27]. Because Dengvaxia™ is a live attenuated vaccine, the components of the vaccine must infect and replicate within the host to induce a protective immune response. However, the four vaccine components of Dengvaxia™ are not equally infective, contributing to its imbalanced humoral response. The DENV-4 component is more infectious than the other 3 components of the vaccine and induces a greater homotypic antibody response. The DENV-4 component was detected by RT-PCR in 44.2% of dengue-naïve subjects following first vaccination compared with only 12.6% for the DENV-3 component, and 7.4% for the DENV-1 component. The DENV-2 component of the vaccine was not detected in any subject following any vaccination [28].

The live attenuated dengue vaccine TV003/TV005 was developed by the U.S. National Institutes of Health. To ensure that each of the components of the vaccine was sufficiently attenuated and immunogenic, numerous clinical trials were conducted of both the individual monovalent vaccine components and different tetravalent admixtures (Table 1). Clinical studies were designed to assess the infectivity, immunogenicity, and reactogenicity of monovalent candidate and tetravalent candidate vaccines. In addition, these studies were used to determine the number of doses needed to induce a suitable neutralizing antibody response, to evaluate the effect of pre-existing flavivirus antibody on the response to the vaccine, the durability of the protective immune response against pseudo-challenge with a second dose of live tetravalent vaccine, and the protection against challenge with DENV-2. The DENV-2 challenge model was developed in direct response to the failure of neutralizing antibody to predict protection against DENV-2 in the Phase 2b study of Dengvaxia™[29]. Several additional challenge studies are in progress but will not be discussed in detail in this review.

All of the monovalent DENV vaccine candidates were developed using recombinant DNA technology. Homologous 30-nucleotide deletions were introduced into 3′ untranslated regions (UTR) of rDEN1 Western Pacific (rDEN1 30) [30], rDEN2 Tonga/74 (rDEN2 30) [31], and rDEN4 Dominica (rDEN4 30) [32] to attenuate these viruses. Two chimeric candidate vaccines were created on the rDEN4 30 background by replacing the prM and E coding sequences of rDEN4 30 with those of DENV-2 NGC (rDEN2/4 30) [33] or DENV-3 Sleman/78 (rDEN3/4 30) [34]. Two additional DENV-3 candidate vaccines were created by adding 30- and 31-nucleotide deletions into the 3′ UTR of rDEN3 Sleman/78 (rDEN3 30,31) or by swapping the entire 3′UTR of rDEN4 30 with that of rDEN3 Sleman/78 (rDEN3–3′D4 30) [34]. The rDEN4 30 candidate vaccine was further modified by the introduction of mutations into nonstructural protein 5 (NS5), designated rDEN4 30–200,201) [35] or into NS3, designated rDEN4 30–4995 [36]. The infectivity of each

component was evaluated by determining the titer of vaccine virus recovered from the blood of volunteers at multiple time-points post-vaccination, as described in the cited references. Nine different monovalent DENV vaccine candidates were evaluated to assess their infectivity and safety profile. Because they were administered as individually, infectivity could be assessed by recovery of infectious virus and immunogenicity as measured seroconversion to the wildtype parent virus determined by the $PRNT_{60}$ assay (Table 2). These studies eliminated several monovalent vaccine candidates from consideration because they were insufficiently infectious (rDEN3/4 30) or thought to be under-attenuated when administered as monovalent vaccines (rDEN2 30 and rDEN4 30–4995). To further assess the safety of the recombinant DENV vaccine candidates, the transmissibility of the rDEN4 30 was assessed early in the vaccine development program. Volunteers were administered a dose of rDEN4 30 100-fold higher than the dose eventually selected for the tetravalent vaccine. Mosquitoes were fed on these volunteers 7, 8, and 9 days after vaccination; days on which volunteers had previously been shown to be viremic. rDEN4 30 was not recovered from more than 300 mosquitoes that had fed on volunteers during this period of expected viremia [37].

Six monovalent vaccines (rDEN1 30, rDEN2/4 30, rDEN3–3′D4 30, rDEN3 30/31, rDEN4 30, and rDEN4 30,31 were then evaluated in 5 different tetravalent admixtures (Table 3). Each of admixtures was well tolerated; a mild maculopapular rash was the only adverse event that occurred significantly more frequently in vaccine recipients than placebo-recipients [38]. Samples were collected approximately every other day post-vaccination through Study Day 16 to measure infectious viremia of each component of each admixture. The rDEN3 30,31 and the DEN4 30 components were more infectious in the tetravalent admixtures than the rDEN3–3′D4 30 and DEN4 30–200,201 components, respectively [38]. rDEN3–3′D4 30 was recovered from20% of subjects who received TV001 but was not recovered from any subject who received TV002. In contrast, rDEN3 30,31 was recovered from 40%, 75%, and 40% of subjects vaccinated with TV003, TV004, or TV005, respectively. rDEN4 30–200,201 was recovered from 5% of subjects vaccinated with TV002 but was not recovered from any subject vaccinated with TV004. In contrast, rDEN4 30 was recovered from 40%, 25%, and 45% of subjects vaccinated with TV001, TV003, and TV005, respectively. Differences in the infectivity of the four vaccine components affected the balance of the neutralizing response to the different serotypes, as shown in Table 4. The immune response to DENV-4 is immunodominant in TV001, likely due to the poor infectivity of rDEN3–3D4 30. However, the DENV-1 response is immunodominant in TV002 because of the poor infectivity of rDEN4 30–200,201. TV003 and TV005 induced the most balanced immune response of the five admixtures. The only difference between TV003 and TV005 is the dose of the rDEN2/4 30 component (10^3 PFU in TV003 and 10^4 PFU in TV005). The dose of the DENV-2 component was increased 10-fold in TV005 because the human infectious dose 50% (HID_{50}) of DEN2/4 30 was higher (10 PFU) than rDEN1 30, rDEN3 30/31, and rDEN4 30 (<10 PFU) as determined in dose ranging studies [39]. TV005 induced seroconversion to DENV-2 in 80% of vaccinated subjects compared with 50% for TV003 (Table 4).

The safety, infectivity, and immunogenicity of TV003 and TV005 were further evaluated head-to-head in a placebo controlled double-blind study, CIR279 [40]. The clinical,

virologic, and serologic responses to TV003 and TV005 in flavivirus-naïve healthy adults are presented in Tables 5, 6, and 7 [38,40]. The data for each admixture from CIR268 and CIR279 were combined for this analysis to provide a larger pool for comparison. In addition, the neutralizing antibody data for these later studies was calculated as the $PRNT_{50}$. Neutralizing antibody data from CIR268 was recalculated as a $PRNT_{50}$ for these comparisons. Both admixtures were well tolerated by volunteers with a mild, maculopapular rash being the only adverse event that occurred significantly more frequently in vaccine recipients compared with placebo recipients (61.7% of both TV003 and TV005 recipients. All four components of both TV003 and TV005 are highly infectious; 75% of vaccinated volunteers had one more infectious vaccine components recovered post-vaccination. In addition, the infectivity of the four components is balanced, particularly the DENV-1, DENV-3, and DENV-4 components. The infectivity of the DENV-2 component improved with the 10-fold higher dose contained in TV005; 22% of TV005 vaccinated individuals had infectious rDEN2/4 30 recovered post-vaccination compared with 7% of TV003 recipients. This was also reflected in the proportion of TV005 recipients who seroconverted to DENV-2 following a single dose (86% compared with 64% of subjects who received TV003. The geometric mean peak neutralizing antibody titers induced by the two different admixtures were not significantly different (Table 7). A higher proportion of TV005 recipients developed a tetravalent neutralizing antibody response following a single dose (79.7% vs 65.5%) however, the trivalent or better response was nearly indistinguishable between the two admixtures; 94.8% of TV003 recipients developed a trivalent or better response compared to 95.0% of TV003 recipients.

Results from the Phase 2b trial of Dengvaxia™ cast doubt on the ability of neutralizing antibody titers to predict the efficacy of a dengue vaccine. For this reason, we developed a DENV-2 challenge model to evaluate the protective efficacy of TV003. We chose to develop a DENV-2 challenge model because rDEN2/4 30 has the highest HID_{50} of the four vaccine components and a lower proportion of TV003 seroconverted to DENV-2 compared with the other 3 serotypes. We repurposed the under-attenuated DENV-2 rDEN2 30 to use as the challenge virus. rDEN2 30 is an American strain of DENV-2 (DEN2/Tonga78) whereas the DENV-2 parent virus of rDEN2/4 30 is an Asian strain. rDEN2 30 infected 100% of flavivirus-naïve healthy adults and induced a mean peak titer ~ 100-fold higher than the vaccine candidate rDEN2/4 30 (2.5 $\log_{10}$ PFU/mL compared to 0.5 $\log_{10}$ PFU/mL [41]. Forty-eight flavivirus subjects were enrolled in the challenge study (CIR287); 24 received TV003 at day 0 and 24 received placebo.

Six months later, 41 subjects were challenged with 10^3 PFU rDEN2 30 (21 had previously received TV003 and 20 had previously received placebo. TV003 induced DENV-2 neutralizing antibody in all vaccinated subjects post-vaccination, although the $PRNT_{50}$ had declined to <1:5 in some subjects by Study Day 180. Infectious DENV-2 challenge virus was recovered from all challenged placebo recipients, however rDEN2 30 could not be recovered by culture or by nucleic acid testing (standard PCR and digital drop PCR) from any vaccinated subject [42]. This study provided conclusive evidence that TV003 is highly protective against DENV-2. Although challenge virus was not recovered from any vaccinated subject, 9 subjects did demonstrate a boost in the DENV-2 $PRNT_{50}$, as defined as

a 4-fold rise in titer, post-challenge; 12 subjects did not mount a 4-fold rise in antibody titer indicative of a sterilizing immune response.

The induction of homotypic neutralizing antibody is thought to be critical for vaccine-induced protection. Because the $PRNT_{50}$ assay utilizes cell lines that do not express $Fc\gamma$ receptors, the assay measures neutralizing antibody only and does not account for the effects of non-neutralizing antibody. To better assess the overall effect of neutralizing and non-neutralizing antibodies in a single assay, we engineered Vero cells to express CD32a, the $Fc\gamma2a$ receptor and compared the $PRNT_{50}$ in those cells with the $PRNT_{50}$ performed with standard Vero cells [43]. Although the $PRNT_{50}$ titers were lower in Vero-CD32a cells (Figure 1), enhancement of infection was not observed in samples post-vaccination or post-challenge in the net neutralization assay (data not shown). In addition, challenge with DENV-2 did not induce a boost in DENV-2 antibody titer, also suggesting strong protection against infection with the DENV-2 challenge virus. These data suggest that the majority of DENV-2 antibody induced by TV003 is homotypic and neutralizing; cross-reactive, non-neutralizing antibody did not abrogate the neutralizing effect of the DENV-2 antibody induced by TV003.

The ability of TV003 to protect flavivirus-naïve volunteers from DENV-2 challenge was critical to the decision of the Instituto Butantan to choose the TV003 formulation dengue vaccine development program. Butantan licensed TV003 and has completed its Phase 2 trial of the vaccine, Butantan-DV. Butantan-DV was well-tolerated and highly immunogenic in flavivirus-naïve and flavivirus-experienced volunteers in Brazil [44]. The vaccine is currently in Phase 3 clinical trial in Brazil with efficacy results expected to be released in 2021. In addition, 3 manufacturers in India have licensed the vaccine for in-country manufacture and Merck & Co. has licensed the vaccine for its dengue-development program. Given the billions of people currently at risk for dengue, it is hoped that multiple manufactures will be able to produce the vaccine to meet the needs of those in dengue-endemic areas.

In summary, an iterative approach was taken was to ensure that each component of the NIH live attenuated vaccine had an acceptable safety profile and was able to infect and replicate in the host post-vaccination. The ability to infect and replicate is critical for these live attenuated vaccines to ensure that each will induce a homotypic antibody response and reduce the risk for enhanced disease upon subsequent exposure to wild-type dengue. TV003 has demonstrated balanced infectivity and a balanced humoral and cellular immune response. Although these features suggest the vaccine will induce protection against dengue, only the Phase 3 efficacy study will definitively evaluate this.

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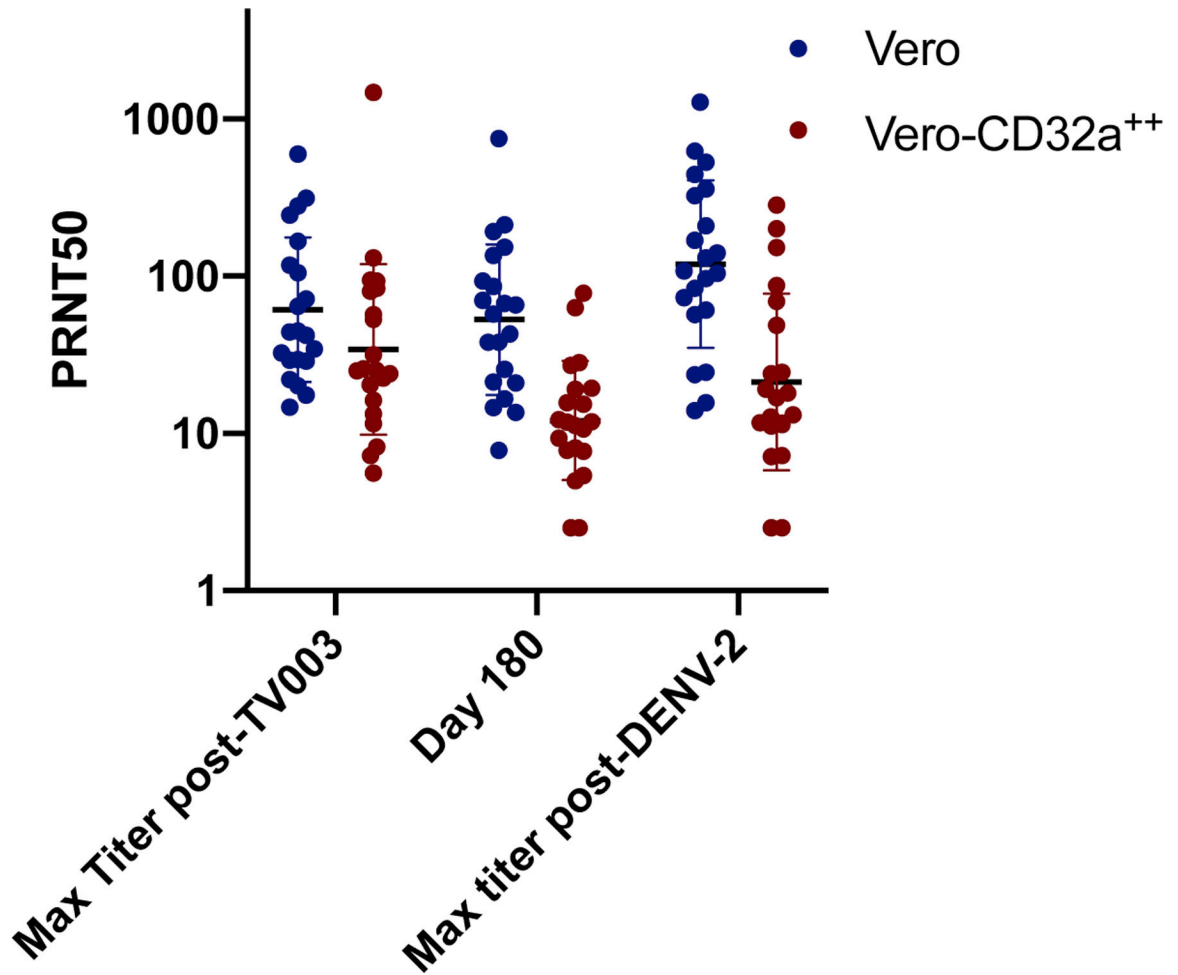
Figure 1:
The neutralizing antibody titers induced by TV003 in CIR287 as measured in Vero cells (Blue) and Vero cells expressing CD32a (Vero-CD32a, Red). Neutralizing antibody titers were measured at study days 0, 28, 56, 90 post-vaccination and challenge. The study day 180 titer is the day of challenge. Seroconversion was defined as 1:10 post-vaccination or 4-fold rise in titer post-challenge. All subjects were flavivirus-naïve prior to challenge ($PRNT_{50} < 5$).

Table 1:

Clinical trials of the NIH live attenuated tetravalent dengue vaccine and its components

Vaccine	# vaccinees	# placebo recipients	Clinicaltrials.gov	Reference
DEN4 30	20	0	Not available	Durbin 2001[32], Troyer 2001[37]
DEN4 30	80	16	Not available	Durbin, 2005 [45]
DEN2/4 30	20	8	Not available	Durbin, 2006 [46]
DEN1 30	20	8	NCT89908	Durbin, 2006 [47]
DEN4 30–200,201	40	16	NCT270699	McArthur, 2008 [48]
DEN4 30–4995	20	8	NCT322946	Wright, 2009 [49]
Heterologous vaccination	30	6	NCT458120	Durbin, 2011 [50]
DEN3/4 30	40	16	NCT375726	Durbin, 2011 [39]
DEN1 30 2 doses	50	10	NCT473135	Durbin, 2011 [51]
DEN2/4 30–2 doses	20	5	NCT920517	Durbin, 2011 [39]
DEN3–3'D4 30	20	8	NCT712803	Durbin, 2011 [39]
DEN1 30-low dose	15	3	NCT1084291	Lindow, 2012 [52]
DEN2/4 30-low dose	15	3	NCT2317900	Durbin, 2011 [39]
DEN4 30	50	20	NCT919178	Durbin, 2011 [39]
DEN3 30/31	60	20	NCT831012	Durbin, 2011 [39]
TV001, TV002, TV003, TV004	100	40	NCT01072786	Durbin, 2013 [38]
TV003/TV005	80	32	NCT01436422	Kirkpatrick, 2013 [40]
TV003	40	16	NCT01506570	Whitehead, 2017 [53]
TV003	40	8	NCT01782300	Durbin, 2016 [54]
DEN2 30–7169	10	4	NCT01931176	Larsen, 2015 [41]
TV003 + DEN2 30 challenge	24	24	NCT02021968	Kirkpatrick, 2016 [42]

Table 2:

Infectivity of monovalent dengue vaccine candidates

Virus	Dose (log$_{10}$ PFU)	No. subjects	% viremic	Mean peak virus titer log$_{10}$ PFU/mL	% seroconverting
rDEN1 30	3	71	61	1.0	93
rDEN2/4 30	3	40	60	0.5	100
rDEN2 30	3	10	100	2.5	100
rDEN3/4 30	3	20	15	1.0	30
rDEN3/4 30	4	20	0	n/a	25
rDEN3 30/31	3	50	34	0.5	81
rDEN3–3'D4 30	3	20	20	0.6	80
rDEN4 30	3	50	26	0.7	93
rDEN4 30–200,201	5	20	0	n/a	100
rDEN4 30–4995	5	20	0	n/a	95

Table 3:

Tetravalent admixtures evaluated in clinical trial

	Administered dose of each component (log$_{10}$ PFU)	Monovalent dengue vaccine component			
		DENV-1	**DENV-2**	**DENV-3**	**DENV-4**
TV001	3,3,3,3	rDEN1 30	rDEN2/4 30	rDEN3–3'D4 30	rDEN4 30
TV002	3,3,3,3	rDEN1 30	rDEN2/4 30	rDEN3–3'D4 30	rDEN4 30–200,201
TV003	**3,3,3,3**	**rDEN1 30**	**rDEN2/4 30**	**rDEN3 30,31**	**rDEN4 30**
TV004	3,3,3,3	rDEN1 30	rDEN2/4 30	rDEN3 30,31	rDEN4 30–200,201
TV005	**3,4,3,3**	**rDEN1 30**	**rDEN2/4 30**	**rDEN3 30,31**	**rDEN4 30**

[1.]Bolded formulations are those chosen to move forward for further evaluation

Table 4:

Immunogenicity of tetravalent admixtures

| Admixture | No. subjects | % Seropositive (PRNT$_{60}$ 10) | | | | Geometric mean peak titer, reciprocal, PRNT$_{60}$ | | | |
		DENV-1	DENV-2	DENV-3	DENV-4	DENV-1	DENV-2	DENV-3	DENV-4
TV001	20	80	65	60	85	54	39	36	154
TV002	20	80	60	75	90	118	41	31	32
TV003	20	100	50	85	100	62	44	36	65
TV004	20	75	50	85	100	36	17	124	32
TV005	20	90	80	90	100	44	41	42	80

Table 5:

Clinical response to Dose 1 of TV003 or TV005 (CIR268 & 279 and combined)

Adverse event	Treatment group					
	TV003 (n=60)	Placebo (n=48)	p value (1-sided)[2]	TV005 (n=60)	Placebo (n=48)	p value (1-sided)[3]
Injection site:						
Erythema	4.8%	4.2%	0.6784	5.0%	4.2%	0.6784
Pain	0.0%	8.3%	1.0000	3.3%	0.0%	0.5077
Tenderness	5.0%	4.2%	0.6784	1.7%	4.2%	0.9208
Induration	3.3%	0.0%	0.5077	1.2%	0.0%	0.7143
Systemic:						
Fever	0.0%	0.0%	n/a	3.3%	0.0%	0.5077
Headache	36.7%	29.2%	0.3488	51.7%	41.7%	0.2792
Rash	**61.7%**	**0.0%**	**<0.0001**	**61.7%**	**0.0%**	**<0.0001**
Neutropenia[1]	8.3%	8.3%	0.6833	6.7%	0.0%	0.2527
Elevated ALT	5.0%	0.0%	0.3591	3.3%	4.2%	0.8050
Myalgia	8.3%	8.3%	0.6833	11.7%	4.2%	0.2713
Arthralgia	3.3%	4.2%	0.8050	0.0%	0.0%	n/a
Retro-orbital Pain	6.7%	8.3%	0.7767	6.7%	8.3%	0.7767
Fatigue	16.7%	4.2%	0.1159	28.3%	20.8%	0.3393
Photophobia	0.0%	4.2%	1.0000	5.0%	4.2%	0.6784
Elevated PT	6.7%	12.5%	0.9012	5.0%	4.2%	0.6784
Elevated PTT	6.7%	8.3%	0.7767	0.0%	4.2%	1.0000
Thrombocytopenia	0.0%	0.0%	n/a	0.0%	0.0%	n/a

[1] Neutropenia was defined as an ANC 1,000/mm^3 in CIR279. Neutropenia was defined as an ANC 1,500/mm^3 for CIR268.

[2] Probability is greater for TV003 vs placebo

[3] Probability is greater for TV005 vs placebo

Table 6:

Viremia induced by TV003 and TV005 (replication in tissue culture)

Admixture	Vaccine components	No. Subjects 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 (N=60)	DEN1 30	15 (25)	0.6 ± 0.1	1.4	10.9 (8 – 15)	2.1 (1 – 5))
	DEN2/4 30	4 (7)	0.5 ± 0.0	0.5	7.5 (6 – 10)	1.0 (all 1 day)
	DEN3 30,31	23 (38)	0.6 ± 0.0	1.2	9.3 (5 – 14)	2.5 (1 – 7)
	DEN4 30	18 (30)	0.5 ± 0.0	0.7	9.3 (6 – 16)	1.9 (1 – 6)
	Total Viremic	45 (**75**)				
TV005 (N=60)	DEN1 30	17 (28)	0.6 ± 0.1	1.7	12.3 (8 – 16)	2.2 (1 – 7)
	DEN2/4 30	13 (22)	0.5 ± 0.0	0.5	9.1 (2 – 14)	1.8 (1 – 8)
	DEN3 30,31	25 (42)	0.6 ± 0.0	1.0	9.2 (6 – 14)	2.8 (1 – 9)
	DEN4 30	18 (30)	0.6 ± 0.1	1.5	10.1 (6 – 16)	2.2 (1 – 10)
	Total Viremic	46 (**77**)				

Table 7:

Cumulative serologic response following a single dose of TV003 or TV005 in flavivirus-naïve subjects

	% seroconverted			
	DENV-1	**DENV-2**	**DENV-3**	**DENV-4**
TV003 (N=58[1])	97.0	64.0	98.0	100.0
TV005 (n=59[1])	91.5	86.4	94.9	96.6
	Geometric Mean Peak Titer (Median)[2,3]			
	DENV-1	**DENV-2**	**DENV-3**	**DENV-4**
TV003 (N=82[1])	70.6 (75)	45.2 (50)	68.1 (57)	123.3 (133)
TV005 (n=59[1])	54.4 (43)	89.3 (101)	80.6 (78)	164.7 (172)
	Percent of TV005 recipients with indicated multivalent response (cumulative)			
	TETRA	**TRI**	**BI**	**MONO**
TV003 (N=82[1])	65.5	29.3 (94.8)	5.2 (100)	0 (100)
TV005 (n=59[1])	79.7	15.3 (95.0)	1.7 (96.7)	3.3 (100)

[1.] From CIR268 and CIR279

[2.] Based on $PRNT_{50}$. Seropositive response is defined as a $PRNT_{50}$ 1:10 by Study Day 90.

[3.] $PRNT_{50}$ titer calculated only for those subjects who seroconverted