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PRODUCT REVIEW

A review of Dengvaxia®: development to deployment

Stephen J. Thomas [a] and In-Kyu Yoon[b]

[a]State University of New York, Upstate Medical University, Division of Infectious Diseases, Institute for Global Health and Translational Sciences, Syracuse, NY, USA; [b]Global Dengue & Aedes-Transmitted Diseases Consortium, International Vaccine Institute, SNU Research Park, Gwanak-gu, Republic of Korea

ABSTRACT

Dengue is the world's most prevalent and important arboviral disease. More than 50% of the world's population lives at daily risk of infection and it is estimated more than 95 million people a year seek medical care following infection. Severe disease can manifest as plasma leakage and potential for clinically significant hemorrhage, shock, and death. Treatment is supportive and there is currently no licensed anti-dengue virus prophylactic or therapeutic compound. A single dengue vaccine, Sanofi Pasteur's Dengvaxia®, has been licensed in 20 countries but uptake has been poor. A safety signal in dengue seronegative vaccine recipients stimulated an international re-look at the vaccine performance profile, new World Health Organization recommendations for use, and controversy in the Philippines involving the government, regulatory agencies, Sanofi Pasteur, clinicians responsible for testing and administering the vaccine, and the parents of vaccinated children. In this review, we provide an overview of Dengvaxia's® development and discuss what has been learned about product performance since its licensure.

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Introduction

Dengue is an increasing public health threat

Dengue is a continuously increasing global public health threat with the four dengue virus types (DENV-1 to 4) now co-circulating in most dengue endemic areas.[1] Population growth, an expansion of areas hospitable for *Aedes* mosquito species, and the ease of travel have all contributed to a steady rise in DENV infections and disease.[2] It is estimated there are over 390 million infections globally each year of which more than 95 million are clinically apparent.[3] There is no specific anti-DENV therapeutic, but supportive care is very effective when delivered by experienced practitioners.

DENV infections may be asymptomatic, result in a mild and non-specific febrile illness, cause classic dengue fever, or, in a small percentage of individuals, result in a severe disease phenotype. Severe disease manifests most often with plasma leakage but may also include clinically significant bleeding; the potential end result of both is decreased intravascular volume, decreased organ perfusion, and the potential for shock and death. It is not completely understood why some people experience no disease and others severe disease, but there is strong evidence sequential infections with different DENV types separated by more than 18 months significantly increases the risk for a severe disease outcome.[4-7] Four antigenically distinct DENV types (DENV-1 to −4) cause dengue. Infection with one type confers long-lasting homotypic protection and short-term heterotypic protection. DENV infection triggers an immune response that can result in protection or disease enhancement during subsequent heterotypic infections, thus complicating the effort to develop dengue vaccines.[8] A variety of factors including viral characteristics, host immunity and genetics, and epidemiological context, along with the relative timing of these factors, play a role in ultimately protecting against or enhancing disease.[9] In order to address this problem, vaccine developers have primarily sought to induce simultaneous tetravalent immunity against all four DENV types. However, these efforts have been hampered by an incomplete understanding of the relevant immune responses that contribute to protection or enhancement.

Dengue vaccine development landscape

A safe and efficacious dengue vaccine capable of preventing clinically significant disease caused by any of the DENV types will be needed as part of a comprehensive global prevention and control strategy. Reaching this goal, however, has been difficult and success has eluded vaccine developers for nearly 75 years.[10] In addition to the requirement to successfully develop a vaccine for each DENV type and then combine them, there have been other development challenges such as the absence of a well-characterized animal model of disease which recapitulates human immunopathology and the absence of an immune correlate or surrogate of protection.

The only licensed dengue vaccine is Sanofi Pasteur's Dengvaxia®, which has now been registered in 20 dengue endemic countries, and more recently by European Union (EU) and United States (US) regulatory authorities. However, immunization implementation has been limited to

CONTACT Stephen J. Thomas thomstep@upstate.edu Upstate Medical University, State University of New York, 725 Irving Ave., Suite 311, Syracuse, New York 13210, USA.

subnational public health programs in only two countries, Brazil and the Philippines. Low vaccine uptake has been fueled by concerns about the increased risk of severe dengue in vaccinated dengue seronegative individuals and vaccine affordability.[11] Dengvaxia® development, deployment, and long-term safety experiences provide important lessons for dengue vaccine and anti-DENV therapeutic development and use.

Two other dengue vaccines are currently in phase 3 trials. Takeda's dengue vaccine candidate, TAK-003, is being evaluated in a multi-country phase 3 trial (NCT02747927) in Asia and Latin America, while Instituto Butantan's dengue vaccine candidate, Butantan-DV, is being evaluated in a single-country phase 3 trial (NCT02406729) in Brazil. The TAK-003 is based on DENV-2 backbone with DENV-DENV chimeras (DENV-2/-2, DENV-2, DENV-2/-3, and DENV-2/-4). Similarly, Bhutantan-DV is a live virus vaccine attenuated thru directed mutagenesis with one DENV-DENV chimera (DENV-1, DENV-4/-2, DENV-3, and DENV-4). Safety, immunogenicity, and efficacy reports are expected.

The Dengvaxia® experience, which we will detail below, has and will continue to impact subsequent dengue vaccine candidate development efforts, as well as prophylactic and therapeutic compound development. Vaccines and compounds will need to be assessed not only for acute and near-term safety but also for safety remote from vaccination or compound administration. Immune responses imparted by vaccination will be assessed for their propensity to increase the occurrence of symptomatic infection or severe disease in recipients compared to non-recipients. Similarly, the immune profiles developed following a natural infection which is "prevented" or interrupted by compound administration will be under scrutiny. DENV type-specific efficacy will also be of interest as well as efficacy durability in different recipient groups (i.e., seronegative versus seropositive, different age groups). Defining a correlate or surrogate of protection will remain of significant interest and a priority in the dengue vaccine field.

Of note, the information and data detailed below were taken from publications, when available, or Sanofi's briefing document recently provided to the U.S. Vaccines and Related Biological Products Advisory Committee in preparation for their, March 7, 2019 review of Dengvaxia®, or both (https://www.fda.gov/media/120943/download, accessed 31 APR 2019).

Dengvaxia® construct

Dengvaxia® is a live attenuated tetravalent vaccine consisting of chimeras made up of structural pre-membrane (prM) and envelope (E) genes of the four DENV types combined with the nonstructural genes of yellow fever 17D vaccine strain (chimeric yellow fever dengue – CYD). The chimeric approach originated at St. Louis University and was first used to develop a Japanese encephalitis vaccine construct.[12] The chimeric technology was later applied to dengue at Acambis, Inc. which subsequently became part of Sanofi Pasteur.[13] The Dengvaxia® parent strains consist of type 1: Thailand PUO-359/TVP-1140, type 2: Thailand PUO-218, type 3: Thailand PaH881/88, and type 4: Indonesia 1228 (TVP-980).[14] Each monovalent CYD DENV was obtained separately via recombinant deoxyribonucleic acid (DNA) technology. The four chimeric vaccine DENVs were cultured in Vero cells and then combined into a single vaccine formulation.

Phenotypic characterizations demonstrated stable plaque size for each DENV at all production steps. The CYD genomes (DENV-1–4) were fully sequenced at various stages throughout the production of vaccine lots to good manufacturing practice (GMP) standards, from the first passages, to premaster seed lots (PMSL), master seed lots (MSL) and bulk, and ultimately at a later step in the process (bulk + 10 passages).[15] Nine point mutations across all DENV types were identified; five at late passage (p10 – p21), three in a mixed population with the original sequence, and one silent mutation (all except one were in the non-structural (NS) regions). Viral morphology observed by electron microscopy was typical for a flavivirus at different stages of maturation (round, smooth particles of about 52–54 nm and spiked or partially spiked particles of about 54–56 nm).[16,17] The ratio of non-infectious to infectious particles was consistent throughout all production steps.[18,19] Additional studies explored protein content consistency using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, replication potential in insect C6/36 cells, temperature sensitivity, replication in Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin-transfected (DC SIGN) cell lines, and glycosylation status.[18,20-22]

The vaccine is provided in a powder and solvent for suspension containing ~5 log10 cell-culture infectious dose 50% (CCID50) of each live, attenuated, DENV type. Dengvaxia is a sterile and freeze-dried product reconstituted before injection with a sterile solution of 0.4% sodium chloride. The vaccine (freeze-dried product) and the diluent are presented in a single-dose vial. After reconstitution, one dose (0.5 mL) is administered by needle in the subcutaneous (SC) space. Three vaccine doses are provided 6 months apart; 0, 6, and 12 months.

Dengvaxia® is considered a genetically modified organism and risk assessments (humans and environment) have been completed.[23](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-clinical-aspects-live-recombinant-viral-vectored-vaccines_en.pdf, accessed 7 MAY 2019)(https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessments-medicinal-products-consisting-containing-genetically_en.pdf, accessed 7 MAY 2019)(https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonization-technical-requirements-registration-pharmaceuticals-human-use_en-10.pdf)(https://www.who.int/biologicals/Clinical_guidelines_revised_IK_29_Oct_2015.pdf, accessed 7 MAY 2019). The potential for CYD viruses to enter the environment was evaluated by assessing vaccine virus shedding following administration. No safety concerns were associated with viral RNA shedding assessed in urine and saliva. Dengvaxia® contains no adjuvant or preservatives. A proprietary stabilizer is present in the finished product with accelerated stability studies demonstrating vaccine from the phase 3 lots of CYD TDV (single dose presentation) was stable up to 1

month at 25 ± 2°C, and that the viral titer decreased by less than 0.5 log 10 CCID50 after 7 days at +37 ± 2°C. Reconstituted vaccine was found to be stable for up to 6 h at +5 ± 3°C.[18] No material of biological origin (animal or human) is used in the CYD virus seed lot system manufacturing process, Vero cell banking system, or CYD Drug Substance (DS) and Drug Product (DP). Dengvaxia® contains no material of porcine origin. Extensive testing for adventitious agents is completed using *in vivo* animal testing, *in vitro* cell substrate testing, and molecular assessments of the manufacturing process of the seed lots, cell banks and DS. See Table 1 for a summary of relevant non-clinical studies performed during the development of Dengvaxia®.

Preclinical in vitro studies

As an infected female *Aedes* mosquito probes for a blood meal and viral particles are introduced intracutaneously, dendritic cells (DCs) are amongst the first immune cells to encounter the virus. DCs are efficient antigen-presenting cells (APCs) and initiate the immune response cascade following DENV infection or immunization.[38] The CYD viruses were assessed for their infectivity of immature human myeloid DCs revealing they induced DC maturation with a limited inflammatory cytokine response and expression of anti-viral type I interferon (IFN).[21,31] These *in vitro* findings suggested that the vaccine induces a controlled inflammatory response favoring efficient antigen presentation, potential adaptive immunity, and perhaps safety of acute vaccination. See Table 1 for a summary of preclinical *in vitro* studies performed during the development of Dengvaxia®.

Preclinical studies in non-human primates

Rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques can be inoculated with the DENVs resulting in viral replication and a measurable immune response but there is a minimal, if any, clinical disease phenotype produced. Peripheral RNAemia, measured with quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), and viremia, measured by plaque assay, have been used as indicators of human disease severity.[39-46]

A single-dose of tetravalent CYD was administered subcutaneously (SC) to cynomolgus macaques, demonstrating tetravalent neutralizing antibody seroconversion. When followed by SC DENV challenge 6 months later, 22 of 24 monkeys were protected from DENV viremia, although anamnestic antibody response after challenge occurred in most of the NHPs, suggesting possible low-level infection.[34] In another study in cynomolgus macaques, interference among the vaccine DENVs was evaluated in a study that included tetravalent vaccine with equal concentrations of each DENV viral antigen (5 logs of each DENV type), demonstrating DENV-4, and to a lesser extent DENV-1, had a predominant neutralizing antibody response following vaccination.[33] A modified tetravalent formulation with a reduced DENV-4 component (5 logs of DENV-1, −2, −3 and 3 logs of DENV-4) induced a predominant DENV-1 neutralizing antibody response following vaccination.[35]

To evaluate *in vitro* neutralization against different DENV strains, monkey sera collected 2 weeks after Dengvaxia® administration in cynomolgus macaques neutralized a broad range of DENVs representing all types and most genotypes, suggesting a vaccine with potential to protect against a diverse range of circulating strains.[36] This was subsequently not substantiated in human clinical efficacy trials, calling into question the clinical relevance of *in vitro* assays and NHP models. However, the early timing of blood collection after vaccination in this NHP study may have resulted in a mix of homotypic and highly cross-reactive heterotypic antibodies which over-estimated the protective breadth of the vaccine.[47,48]

A more stringent DENV NHP challenge model was explored by administering two-doses of Dengvaxia® to cynomolgus macaques followed by intravenous (IV) challenge with 10^7 CCID50 of DENV 8 months after vaccination. All vaccinated monkeys were protected from RNAemia after DENV-4 challenge, while only 6 of 18 were protected from RNAemia after DENV-2 challenge; the remainder had RNAemia levels 1–3 logs lower than controls.[37] This study suggests that an NHP model with different conditions may have had more clinically relevance. Please see Table 1 for a summary of preclinical *in vivo* studies performed during the development of Dengvaxia®.

Clinical development

Overview

Early clinical studies (phase 1 and 2) are performed to build a vaccine candidate's safety profile and to explore immunogenicity. Taken together, these data build the case for whether a vaccine candidate has the potential for clinical benefit and should proceed to advanced clinical testing (phase 2b and 3).

Dengvaxia® has been studied in 26 clinical trials including more than 41,000 volunteers. At least one injection of final tetravalent formulation has been administered to more than 28,500 individuals from 9 months through 60 years of age and 20,974 individuals aged 9 years through 45 years. Early studies explored safety and immunogenicity in different ages, regions, and flavivirus priming status. Clinical end-point studies were performed in Thailand (phase 2b, CYD23) and Asia (CYD14) and Latin America (CYD15).[49-51] See Table 2 for a summary of clinical trials performed during the development of Dengvaxia®.

Phase 1-3 trial review

A phase 1 trial was performed in the U.S. using a monovalent DENV-2 formulation (CYD01).[52] Subsequent phase 1 trials (CYD02, CYD04, CYD05, CYD06) evaluated the safety of a tetravalent Dengvaxia® in adults from non-endemic areas in the U.S. (CYD02, CYD04) and in a second step in adults and children in non-endemic (CYD06) and endemic areas (CYD05) in Mexico and the Philippines, respectively.,[53-55] The studies were first conducted in non-endemic areas to collect data from individuals who were seronegative to flaviviruses, and especially to dengue, prior to vaccination. These phase 1 studies together with three phase 2 studies (CYD10, CYD11, CYD12) provided data on safety and immune responses induced by several different vaccine formulations

Table 1. Summary of relevant non-clinical studies during development of CYD vaccine.

Purpose	Study authors [ref]	Study design	Findings	Impact
Genetic and phenotypic stability	Pugachev et al. 2004[24]	*In vitro* passage and sequencing of ChimeriVax-DEN types 1–4	- Low nucleotide error rate of yellow fever RNA polymerase	- Supported genetic and phenotypic stability of ChimeriVax-DEN types 1–4
	Barban et al. 2007[25]	Sequencing and assessment of plaque size distribution from 12 bulk lots of yellow fever vaccine produced between 1990 and 2002	- Identical genome sequences of all 12 lots - Homogeneity of viral plaque size distribution	- Supported genetic and phenotypic stability of YF17D-based vaccines
	Mantel et al. 2011[15]	Sequencing of CYD1–4 from different stages of development and manufacturing	- No genetic changes from premaster seed to bulk lots - Few genetic variations beyond bulk lots, but with no effect on plaque size, mouse neurovirulence or reversion	- Supported genetic and phenotypic stability of CYD1-4
Post-translational modifications	Dubayle et al. 2015[20]	Analysis of structure of N-linked glycans on CYD1-4	- Asparagine residues 67 and 153 were glycosylated on CYD1-4	- Glycosylation may be similar between CYDs and wild-type DENVs
Recombination risk	McGee et al. 2008[26]	ChimeriVax-DEN4 YF17D backbone replaced with virulent YFV Asibi strain, then given to *Ae. aegypti*	- Recombination with virulent backbone did not increase transmissibility in mosquito vector	- Low risk with recombination
	McGee et al. 2008[27]	ChimeriVax-DEN4 YF17D backbone replaced with virulent YFV Asibi strain, then injected into cynomolgus macaques	- Recombination with virulent backbone did not reduce attenuation in monkeys	- Low risk with recombination
Transmission risk by mosquito vectors	Johnson et al. 2004[28]	*Ae. aegypti* and C6/36 cells infected by ChimeriVax-DEN1-4	- ChimeriVax-DENs growth in C6/36 cells and *Ae. aegypti* was lower than YF vaccine	- Low risk of mosquito vector transmission
	Higgs et al. 2006[22]	ChimeriVax-DEN1-4 fed to field collected *Ae. aegypti* and *albopictus*	- Low infection and dissemination compared to wild-type DENVs	- Low risk of mosquito vector transmission
Neurovirulence; reversion to virulence	Monath et al. 2005[29]	ChimeriVax-DEN1 or 2 inoculated intracranially into suckling mice and rhesus and cynomolgus macaques	- ChimeriVax-DENs were less neurovirulent than yellow fever vaccine	- No evidence for neurovirulence - No evidence for reversion to virulence - Supported mouse model for neurovirulence evaluation
Non-clinical safety	Ravel et al. 2017[30]	CYD-TDV evaluated for toxicity in cynomolgus macaques	- No toxicological findings - Transient low-level CYD-TDV RNAemia	- Low likelihood of toxicity
In vitro immunogenicity	Brandler et al. 2005[31]	*In vitro* growth kinetics of ChimeriVax-DEN1-4 and parent viruses (wild-type DEN-1-4 and YF 17D) in human myeloid dendritic cells and in 3 hepatic cell lines (HepG2, Huh7, and THLE-3)	- ChimeriVax-DEN1-4 viruses replicated well in dendritic cells, but poorly in 2 of 3 hepatic cell lines	- Supported low likelihood of hepatotropism
	Deauvieau et al. 2007[21]	*In vitro* effects of CYD infection of dendritic cells on activation/maturation and cytokine production	- CYD induced dendritic cell maturation and controlled response with limited inflammatory cytokines	- Controlled inflammatory response supported potential adaptive immunity and perhaps safety of acute vaccine administration
	Balas et al. 2011[32]	DNA microarrays used to assess *in vitro* innate response in CYD-infected human myeloid dendritic cells	- Microarray signature showed type I IFN and associated adaptive response genes	- Controlled inflammatory response supported potential adaptive immunity and perhaps safety of acute vaccine administration

(*Continued*)

Table 1. (Continued).

Purpose	Study authors [ref]	Study design	Findings	Impact
In vivo immunogenicity and/or protection	Guirakhoo et al. 2000[13]	Rhesus macaques immunized with monovalent ChimeriVax-DEN2, then challenged after 62 days with 5.0 log10 FFU of wild-type DENV-2 subcutaneously	- Vaccinated monkeys developed brief vaccine viremia - Protected against viremia from DENV-2 challenge	- Supported further development of ChimeriVax-DENs
	Guirakhoo et al. 2001[14]	Rhesus macaques immunized with monovalent ChimeriVax-DEN1, 3, or 4, or tetravalent ChimeriVax-DEN1-4	- 8 of 9 monkeys immunized with monovalent vaccine, and 6 of 6 with tetravalent vaccine seroconverted after one dose; DENV-2 component in tetravalent vaccine appeared to be immunodominant - 4 of 9 monkeys immunized with monovalent vaccine, then boosted with tetravalent vaccine 6 months later developed low levels of vaccine viremia after 2nd dose	- This was first tetravalent ChimeriVax-DEN formulation evaluated in monkeys - DENV-2 component in tetravalent vaccine was lowered for next monkey study
	Guirakhoo et al. 2002[33]	Cynomolgus macaques received monovalent or tetravalent ChimeriVax-DEN; tetravalent vaccine group boosted after 2 months	- DENV-2 component which was lowered to 3 log10 PFU in tetravalent vaccine, resulted in higher response to DENV-4	- Further adjustments in tetravalent formulations needed
	Guirakhoo et al. 2004[34]	Cynomolgus macaques received single-dose immunization with tetravalent vaccine followed by subcutaneous DENV challenge after 6 months; 4 different tetravalent vaccine formulations were assessed	- Tetravalent high-dose (5/5/5/5 log10 PFU) and low-dose (3/3/3/3 log10 PFU) resulted in tetravalent seroconversion - 22 of 24 monkeys were protected from viremia after challenge, although most had anamnestic antibody response after challenge	- Supported tetravalent formulation adjustments - Suggested likely protection against all 4 DENV types (but contrary to subsequent clinical efficacy trial results) - Supported further testing in humans
	Guy et al. 2009[35]	Cynomolgus macaques received various combinations of vaccines to assess for interference	- DENV-4, and to a lesser extent, DEN-1 were dominant in inducing neutralizing antibodies - Vaccine viremia was observed mainly with DENV-4	- Tetravalent formulation 5/5/5/5 log10 PFU resulted in interference, likely with DENV-4 dominant
	Barban et al. 2012[36]	Sera obtained from previously immunized cynomolgus macaques were tested *in vitro* for neutralization of diverse DENV strains/serotypes	- Sera from monkeys that received tetravalent vaccine neutralized diverse DENV strains/seroytpes of DENVs	- Suggested vaccine-induced antibodies could provide broad coverage (but contrary to subsequent clinical efficacy trial results)
	Barban et al. 2018[37]	Cynomolgus macaques immunized with CYD-TDV were challenged with more "stringent" dose (intravenous 7 log10 CCID50) of wild-type DENV	- 6 of 6 monkeys were protected from DENV-4 challenge, but only 6 of 18 protected from DENV-2 challenge	- Suggested that different monkey models may be more relevant to clinical outcomes

Table 2. Summary of clinical trials of CYD vaccine.

Study code [ref]	Phase	Purpose	Formulation; Schedule	Age Range (years except where months are noted)	Country
CYD01[52]	1	Safety, vaccine viremia, immunogenicity, effect of priming by yellow fever vaccine	Monovalent ChimeriVax™-DEN2 at 3 or 5 log10 PFU; single dose	18–49	USA
CYD02[FDA briefing document, https://www.fda.gov/media/120943/download)]	1	Safety, vaccine viremia, immunogenicity in dengue non-endemic area	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/3.5/12 months	18–45	USA
CYD04[53]	1	Safety, vaccine viremia, immunogenicity in dengue non-endemic area	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/3.5/12 months	18–45	USA
CYD05[54]	1	Safety, vaccine viremia, immunogenicity in dengue endemic area including children, antibody persistence	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/3.5/12 months	2–45	Philippines
CYD06[55]	1	Safety, vaccine viremia, immunogenicity in dengue non-endemic area including children	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/3.5/12 months	2–45	Mexico
CYD08[56]	2	Safety, immunogenicity of concomitant Pentaxim™		12–15 months	Philippines
CYD10[57]	2	Safety, vaccine viremia, immunogenicity, effect of priming by classically attenuated live dengue vaccine	Tetravalent CYD 5/5/5/5 log10 CCID50; single dose	18–40	Australia
CYD11[58]	2	Safety, vaccine viremia, immunogenicity with bivalent and tetravalent formulations in flavivirus-naive adults and those primed by JE vaccine	Bivalent CYD1/3 & 2/4 at 5/5 log10 CCID50; Tetravalent CYD 5/5/5/5 log10 CCID50; 0/105 days	18–45	Mexico
CYD12[59]	2	Formulation comparison	Tetravlent CYD 5/5/5/5 vs 5/5/5/3 vs 4/4/4/4 log10 CCID50; 0/6/12 months	18–45	USA
CYD13[60]	2	Safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–16	Colombia, Honduras, Mexico, Puerto Rico
CYD22[61,62]	2	Safety, immunogenicity, antibody persistence	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	2–45	Vietnam
CYD24[63]	2	Safety, vaccine viremia, immunogenicity in Yellow fever	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	2–11	Peru
CYD28[62,64,65]	2	Safety, immunogenicity, persistence of immune response	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	2–45	Singapore
CYD30[66]	2	Safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–16	Brazil
CYD47[67]	2	Safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	18–45	India
CYD51[68]	2	Immunogenicity in Yellow fever vaccinated subjects	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	18–45	USA
CYD56 [ClinicalTrialss.gov NCT01943825]	2	Immunogenicity with compressed schedule and JE vaccine co-administration	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/2/6 months & 0/6/12 months	18–45	USA
CYD63 [ClinicalTrials.gov NCT02824198]	2	Safety, immunogenicity of booster dose in subjects from CYD28	Tetravalent CYD 5/5/5/5 log10 CCID50; single booster dose	2–45 (at enrollment in CYD28)	Singapore
CYD64[69]	2	Safety, immunogenicity of booster dose in subjects from CYD13 and CYD30	Tetravalent CYD 5/5/5/5 log10 CCID50; single booster dose	9–16 (at enrollment in CYD13 or CYD30)	Brazil, Colombia, Honduras, Mexico, Puerto Rico
CYD65 [ClinicalTrials.gov NCT02628444]	2	Immunogenicity of 1 vs 2 vs 3 dose primary series and booster	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months (with 0, 1 or 2 placebos) followed by single booster dose 1–2 years after 3rd injection	9–50	Colombia, Philippines
CYD23[49,70,71]	2b	Proof-of-concept efficacy, safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	4–11	Thailand
CYD57[70-72]	2b	Long-term safety follow-up of CYD23 subjects	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	4–11 at enrollment in CYD23	Thailand
CYD17[73]	3	Lot consistency	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	18–60	Australia
CYD29[74,75]	3	Safety, immunogenicity of concomitant Yellow fever vaccine	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	12–13 -months	Colombia, Peru
CYD32[76]	3	Safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	2–11	Malaysia
CYD33[77]	3	Safety, immunogenicity of concomitant Pentaxim™	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–12 months	Mexico
CYD66 [ClinicalTrials.gov NCT02992418]	3	Safety, immunogenicity of concomitant tetanus/diphtheria/pertussis vaccine	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–60	Philippines
CYD67 [ClinicalTrials.gov NCT02993757]	3	Safety, immunogenicity of concomitant human papilloma virus vaccine	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–13	Malaysia
CYD71 [ClinicalTrials.gov NCT02979535]	3	Safety, immunogenicity of concomitant human papilloma virus vaccine	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–14	Mexico
CYD14[50,70-72]	3 (efficacy)	Pivotal efficacy, safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	2–11	Indonesia, Malaysia, Thailand, Philippines, Vietnam
CYD15[51,70-72]	3 (efficacy)	Pivotal efficacy, safety, immunogenicity	Tetravalent CYD 5/5/5/5 log10 CCID50; 0/6/12 months	9–16	Brazil, Colombia, Honduras, Mexico, Puerto Rico

and immunization schedules. The results of these studies supported the selection of the final vaccine formulation and schedule: ~5 log10 CCID50 of each live, attenuated, DENV type 1, 2, 3, 4 given as 3 injections 6 months apart.

Additional phase 2 trials testing Dengvaxia® were performed in multiple endemic and non-endemic countries in Asia (India, Philippines, Singapore, Vietnam), Latin America (Brazil, Colombia, Honduras, Mexico, Peru), Australia and the U.S. (CYD13, CYD22, CYD24, CYD28, CYD30), addressing questions related to dose, schedule, priming by other flaviviruses or flavivirus vaccines, and the safety of co-administration of administration of other vaccines.[57-61,63,64,66-68,74] CYD47 was performed in India to assess Dengvaxia's® safety and immunogenicity in Indian populations. A co-administration Phase 2 study (CYD08) was also conducted assessing Dengvaxia® together with measles/mumps/rubella (MMR) vaccine in toddlers less than 2 years of age. An indication for traveler/non-endemic populations was explored (shorter schedule) in a phase 2 adult study in the U.S. (CYD51 and CYD56). In CYD63 and CYD64, a booster dose (5 years after dose three of the primary series) is being evaluated in two phase 2 studies using subsets of individuals who participated in CYD28 and CYD13, respectively. In addition, a study to assess alternate vaccination schedules and booster dose was initiated in individuals 9 to 50 years of age in the Philippines and Colombia (CYD65).

Four phase 3 clinical studies (CYD17, CYD29, CYD32, and CYD33) were performed without assessing for an efficacy clinical endpoint. CYD17 compared lot-to-lot consistency in dengue naïve adults in Australia up to 60 years of age and provided data to support phase 2 to phase 3 bridging required due to new manufacturing processes. A phase 3 trial was conducted in Malaysian children (2–11 years of age) assessing Dengvaxia's® safety and immunogenicity (CYD32). Studies in Peru and Colombia assessed Dengvaxia® co-administration with Yellow fever vaccine in infants and toddlers less than 2 years of age (CYD29), while CYD33 (Mexico) assessed co-administration of DTacP-IPV (diphtheria and tetanus toxoids and acellular pertussis adsorbed and inactivated poliovirus vaccine) as a booster administered with the second injection of Dengvaxia®. Three co-administration studies with human papilloma virus (HPV) vaccine were completed in individuals 9 to 13 years of age in Malaysia (CYD67) and 9 to 14 years in Mexico (CYD71). The third study (Philippines) assessed co-administration of a tetanus/diphtheria/pertussis vaccine in individuals 9 to 60 years (CYD66).[73,75-77]

Safety and immunogenicity summary of non-efficacy studies

Dengvaxia's® acute safety profile was found to be similar to licensed Yellow fever vaccine (YF-VAX®, Sanofi Pasteur, Swiftwater, PA) and not affected by pre-existing Yellow fever immunity. Most volunteers seroconverted in the monovalent DENV-2 trial (CYD01), and pre-existing Yellow fever immunity contributed to a more cross-reactive and enduring anti-DENV antibody response.[52] Subsequent tetravalent studies demonstrated an acceptable acute safety profile with low levels of mostly CYD-4 RNAemia (measured by qRT-PCR) occurring after the first injection. Second and third doses maintained their positive safety profile and demonstrated sequential increases in seroconversion among recipients and increasing anti-DENV geometric mean titers (GMTs).[53] Of interest, exploration of different dosing schedules hinted that a more delayed dosing schedule (more than 4 months between first and second dose) may be superior from an immunogenicity standpoint; an observation made for other live virus dengue vaccine candidates.[78-80] Studies in Mexico, the Philippines, and Australia continued to confirm acute safety in children and adults with varied pre-existing flavivirus immunity, including Japanese encephalitis, at the time of vaccination. The benefit of this pre-existing immunity toward developing rapidly increasing, broad, and potent immune responses after Dengvaxia® administration was also reinforced.[54,55,57]

Efficacy trial (clinical endpoint) review

Three clinical endpoint studies have been conducted with Dengvaxia®; a phase 2b trial in Thailand and two phase 3 trials (CYD14 and CYD15) in Asia Pacific and Latin America.[49-51] Vaccine or control/placebo was administered at study months 0, 6, and 12. The primary efficacy endpoint was protection against dengue disease of any severity caused by any DENV type. The active phase of the study assessing for all symptomatic dengue was completed between study months 0 and 25 while hospital-based surveillance was originally planned from month 25 thru year 6; mid-way thru year 4, the surveillance expansion phase (SEP) was instituted marking a return to active surveillance methods.

Assessment of acute safety and reactogenicity in 9–17-year-olds revealed more vaccine recipients compared to placebo reported expected adverse events (AEs) within 7–14 days following injection, the frequency of grade 3 (severe) reactions was low. Most reactions were mild and resolved within a few days and the frequency of reactions lessened with each subsequent injection. Unsolicited AEs within 28 days after injection were similar between vaccine and control recipients. Serious allergic reactions occurred in <0.1% of vaccine recipients. Serious adverse events (SAEs) thought to be related to the injection was <0.1% in both vaccine and control recipients. Deaths occurred with equal frequency (<0.1%) in both vaccine and control recipients. For those with available baseline dengue serostatus (determined by PRNT50), there was no difference in the frequency or severity of acute adverse events as a function of serostatus. Finally, there were no safety concerns related to vaccine viremia, co-administration of other vaccines, or the inadvertent vaccination of pregnant women; the frequency of adverse pregnancy outcomes in 22 women was similar between vaccine and control recipients.

CYD23 was a Phase 2b proof of concept study in 4 to 11-year-old children residing in Thailand. Subjects were randomized 2:1 to vaccine: placebo, and the per-protocol group for efficacy included 2,452 vaccine and 1,221 placebo recipients. In both vaccine and placebo groups, the mean age of subjects was 8.2 years and the majority (>80%) were between the ages of 6 and 11 years. The male to female ratio was balanced and >90% of subjects were flavivirus seropositive at baseline (dengue = 70.1% and JE/YF = 79.9%). From 28 days following the

last dose of vaccine (injections at time 0, 6 months, 12 months) to the end of the active surveillance phase (study months 0–25), 78 virologically confirmed dengue cases occurred in 77 subjects. The study did not meet the primary efficacy endpoint with an overall efficacy of 30.2% [95% CI: −13.4; 56.6].

CYD23, Dengvaxia's® first clinical endpoint study, yielded important observations; 1) tetravalent dengue vaccines may have variable DENV type-specific efficacy; 2) neutralizing antibody titers may not predict efficacy; and 3) powering a study to assess for DENV type-specific efficacy or efficacy against preventing severe disease would require extremely large sample sizes. Vaccine efficacy following at least one vaccine dose against DENV types −1, −3 and −4 was 61.2%, 81.9%, and 90.0%, with lower bound of the CI above 0, respectively. However, after at least one injection, efficacy against DENV-2 was 3.5%, with lower bound of the CI crossing 0. Considering the low DENV-2 efficacy and the fact most of the dengue cases in the active surveillance period were due to DENV-2 infection (32 cases in the vaccine group compared to 19 in the control), it is not surprising the overall efficacy was low. Neutralizing antibody titers against each DENV type (measured 1 month after dose 3) were well within titer ranges previously hypothesized, and supported by animal and cohort data, to be protective: DENV-1: 146.1 (98.5–216.7); DENV-2: 310 (224–431); DENV-3: 405 (307–534); and DENV-4: 155 (123–196). This observation reinforced concerns about the ability of the PRNT assay to distinguish protective and non-protective, type-specific and cross-reactive antibody responses. Furthermore, wild-type DENV-2 isolates obtained from the CYD23 trial were able to be neutralized in a PRNT assay by sera from CYD-TDV recipients from the trial, further calling into question the clinical relevance of the assay.[81] Nevertheless, in an NHP study in which higher doses of DENV challenge were administered intravenously, NHPs vaccinated with CYD-TDV had poor protection against DENV-2, suggesting that other models may potentially be more clinically relevant.[37] Finally, there were five severe disease cases reported (three in vaccine group and two in control) without any discernable differences in virologic or clinical determinants of severity.[49]

CYD14 and 15 were clinical end-point efficacy studies conducted in five Asia Pacific countries (Philippines, Thailand, Indonesia, Malaysia, and Vietnam) and five Latin American countries (Brazil, Colombia, Honduras, Mexico, and the U.S.[82]). Subjects 2–14 years (N = 10,275) and 9–16 years of age (N = 20,869) were enrolled in CYD14 and 15, respectively. Randomization was 2:1 (vaccine: placebo), equating to 6,851 subjects receiving Dengvaxia® in CYD14 and 13,920 in CYD15. Immunogenicity and reactogenicity subsets were 20% (N = 2000) in CYD14 and 10% (N = 2000) in CYD15. In CYD14 (per-protocol analysis set for efficacy), the mean age of subjects was 8.8 years with a 48% to 52% split between boys and girls, respectively. In the immunogenicity subset, the seropositive status of subjects for dengue or Japanese encephalitis was 79% and 77% for the vaccine and control groups, respectively (dengue alone was 68% and 67%).[50] In CYD15 the mean age was 12.4 years in the per-protocol analysis set for efficacy. The male-to-female split was 49.7% and 50.3%, respectively. In the immunogenicity subset, baseline seropositivity to dengue (any serotype) was 80.6% in the vaccine group and 77.0% in the control group.[51]

Both studies met the primary efficacy endpoint (2–16 years old, after three injections, during active surveillance months 13–25) with an efficacy in CYD14 of 56.5% (43.8–66.4) and CYD15 of 60.8% (52.0–68.0); the combined study efficacy endpoint (CYD14 + 15) was 59.2% (52.3–65.0). Vaccine efficacy in the same population from the first injection (months 0–25) was very similar. Combining studies, DENV type-specific efficacy was greatest for DENV-4 [76.9% (69.5–82.6)], followed by DENV-3 [71.6% (63.0–78.3)] and DENV-1 [54.7 (45.4–62.3)], with the lowest efficacy against DENV-2 [43.0% (29.4–53.9%)]. Efficacy against hospitalized dengue due to any of the DENV types after the first injection (months 0–25) was 67.4% (50.6–78.7) for CYD14 and 80.3% (64.7–89.5) for CYD15, with a combined efficacy of 72.7% (62.3–80.3). Efficacy against severe dengue after the first injection was higher in CYD15 [95.5% (68.8–99.9)] than CYD14 [70.0% (35.7–86.6)], and the combined efficacy was 79.1% (60.0–89.0).

The relative risk (RR) of hospitalized dengue due to any DENV type in CYD14 favored Dengvaxia® during the active study phase (years 1 and 2) and for the entire study period, but was inconclusive for years 3 and 4 as the upper limit of the RR confidence intervals crossed 1. The results were somewhat different in CYD15 with more convincing RRs for the active phase [0.197 (0.11–0.35)] and entire study period [0.323 (0.22–0.47)]. Years 3, 4, and 5 all had RRs below 1, but the upper limit of the CIs crossed 1; 1.16, 1.05, and 4.04, respectively. The RRs of experiencing severe disease conclusively favored Dengvaxia® in CYD14 only for the active phase [0.300 (0.13–0.64)]. In year 3, the data strongly favored the control with a RR of severe disease of 5.497 (0.80–236.60). In CYD15, the data favored the vaccine during the active phase and the entire study period; while the year 3 safety signal was not observed, the upper range of the CI did cross zero [0.300 (0.05–1.54)].

Safety and immunogenicity summary of efficacy studies

In three clinical endpoint studies, Dengvaxia® maintained the positive acute safety and reactogenicity profile established in early clinical studies. DENV type-specific and mean tetravalent neutralizing antibody responses were superior to placebo/control, moderate in titer, and relatively balanced across the different DENV types, but were not directly associated with DENV type-specific efficacy (i.e., immunogenicity by PRNT for a certain type did not predict type-specific efficacy). Vaccine efficacy against any dengue, of any severity, caused by any DENV type was low to moderate, with DENV-4 and −3 efficacy superior to DENV-1 and −2. Efficacy against hospitalized and severe dengue was superior when compared to prevention of any dengue. Efficacy as a function of time from injection demonstrated a positive trend toward the vaccine in years 0–2 and overall (0–5 years), but there was a safety signal in year 3 among some vaccine recipients.

Exploring Dengvaxia's® variable safety and efficacy by age

Dengvaxia® performed differently, from both a safety and efficacy perspective, in different populations. Based on the available data from CYD23, 14, and 15, Sanofi focused on two lines of thinking regarding these observations; 1) there was an age-specific effect, and 2) baseline dengue serostatus at the time of vaccination impacted outcome following natural infection. It was difficult to complete these analyses for a number of reasons; 1) the CYD23, 14, and 15 efficacy studies enrolled subjects across different age ranges limiting age-specific sample size; 2) baseline blood samples were not collected on all subjects making defining baseline serostatus a challenge; 3) surveillance for dengue following injection varied from actively capturing all cases to passively capturing only hospitalized cases; and 4) severe dengue occurs in a small minority of infected individuals (2–4% of second infections) reducing the power and confidence of safety and efficacy calculations related to hospitalized and/or severe disease outcomes. Notwithstanding these challenges, clear and significant trends emerged regarding Dengvaxia's® performance when these factors were analyzed.

In the original manuscript describing the results of long-term follow-up, the RR for dengue-related hospitalization during year 3 of CYD14 was 1.04 (0.52–2.19) for all participants, 7.45 (1.15–313.80) for 2–5-year-olds, 0.63 (0.22–1.83) for 6–11-year-olds, and 0.25 (0.02–1.74) for those 12–14 years of age.[72] When bundling those <9 years of age and those = />9, the RR was 1.58 (0.61–4.83) and 0.57 (0.18–1.86), respectively. CYD15 only enrolled subjects greater than 9 years of age, and the RR for all participants was 0.53 (0.25–1.16). The CYD23 study volunteers had long term follow-up under study number CYD57. Year 3 and 4 data were available for analysis, demonstrating a RR of 1.01 (0.47–2.30) and 0.44 (0.22–1.00) for all participants in year 3 and 4, respectively. When bundling those <9 years of age and those = />9, the RR in years 3 and 4 for those <9 was 1.57 (0.60–4.80) and 0.54 (0.23–1.29), respectively. For those = />9, the RR was 0.31 (0.05–1.58) and 0.31 (0.05–1.58) for years 3 and 4, respectively. DENV type-specific efficacy bundled by participants 9 years of age and older compared to those under 9 years of age across CYD14, 15, and combined 14 + 15 demonstrated moderate efficacy against all DENV types with a combined efficacy of 65.6% (60.7–69.9). Combined (CYD14 + 15) DENV type-specific efficacy ranged from DENV-4 at 83.2% (76.2–88.2) to DENV-2 at 47.1% (31.3–59.2). Combined study efficacy against hospitalization and severe disease defined by the Independent Data Monitoring Committee (IDMC) or 1997 World Health Organization (WHO) criteria in those 9 years of age or greater was 80.8% (70.1–87.7), 93.2% (77.3–98.0), and 92.9% (76.1–97.9), respectively. In those younger than 9 years, the data were less convincing with efficacy against hospitalization of 56.1% (26.2–74.1) and efficacy against severe disease of 44.5% (IMDC) to 66.7% (WHO) with large CIs crossing zero.

In summary, there appears to be an age effect on the safety and efficacy of Dengvaxia®. Specific observations would include; 1) younger vaccine recipients (<9 years of age) appear to experience lower overall vaccine efficacy; 2) younger recipients appear to experience reduced benefit related to prevention of hospitalized and/or severe disease; 3) in the younger vaccine recipients, there was a safety signal of an increased RR of hospitalized and/or severe disease compared to control/placebo recipients; and 4) the above trends peaked at study year 3 and then declined over years 4 and 5.

It is unclear whether the trends described above are related to age as a surrogate marker for some other risk factor (i.e., age-specific serostatus), or whether the independent combination of age and another factor is at play. Clinical epidemiologic studies have identified age as a factor independently associated with hospitalized and/or severe disease, but numerous, similarly designed, studies failed to replicate the finding.[83,84] The hypothesis that age-related differences in physiology may predispose an individual to a higher risk of plasma leakage and severe disease has been suggested.[85,86]

Exploring Dengvaxia's® variable safety and efficacy by serostatus

The vast majority of severe DENV infections occur in individuals who experience sequential DENV infections with different DENV types separated in time by more than 18 months.[4-7] There are numerous hypotheses postulating the causes of severe dengue, many of which are focused on how pre-existing dengue immunity from a first infection triggers an immunopathologic cascade following a second, sequential infection.[87-91] The exact mechanism(s) have not been completely or conclusively explained, but the data supporting the risk of sequential infections are robust. How these theories relate to Dengvaxia's® performance may be found in the observations of how baseline serostatus impacted vaccine safety and efficacy.

As discussed above, only a relatively small number of baseline blood samples were available from trial subjects, making it difficult to assess serostatus as a factor in vaccine performance using only actual measurements. To mitigate this situation, the vaccine Sponsor (Sanofi Pasteur, Lyon, France) used measured (when values were available) or imputed (when values were missing) PRNT50 titers with imputation conducted using covariates to include data generated from study month 13 blood samples (i.e., 1 month after dose 3) tested with a newly developed dengue anti-non-structural protein 1 (NS1) assay.[92] Because the vaccine construct does not contain dengue NS1 protein, the absence of anti-NS1 antibodies from study month 13 samples would indicate the individual had not experienced a natural DENV infection (i.e., had seronegative status).[70]

The Sponsor re-assessed the risk of hospitalization and severe disease in subjects 9–16 years of age, according to baseline serostatus. Data were pooled from CYD23 (57), 14 and 15 including subjects who received at least one dose of vaccine. Regardless of the analytic method (measured, imputed, or NS1), seropositive subjects had a RR of hospitalized or severe dengue which favored the vaccine with RRs ranging from 0.19 to 0.21 for hospitalized disease and 0.15 to

0.18 for severe disease. In the seronegative group, the RR favored the control with RR ranges for hospitalized and severe disease of 1.41–1.51 and 1.41–6.25, respectively; all CIs crossed 1 and were wide in the severe disease analysis. When the RR of hospitalization and severe disease was assessed in 2–8-year-olds based on serostatus following similar parameters (at least one dose, pooled data), there was a trend toward a vaccine benefit in the seropositive vaccine recipients with RR ranging from 0.35 to 0.50 for hospitalization and 0.45 to 0.58 for severe disease; the CIs crossed 1 in this group regardless of the analytic method. In the seronegative group, the RRs favored the control group with a RR range for hospitalization of 1.95–2.48, and for severe disease of 3.31–4.31; many of the CIs in these analyses also crossed 1.

Expanding the age range to include older children (2–16 years) demonstrated a more positive vaccine effect in the seropositive subjects with a range of RR of 0.25–0.32 for hospitalized dengue and 0.27–0.33 for severe dengue with more narrow CIs which did not cross 1. Control continued to be favored over vaccine in the seronegative group with RR ranges for hospitalization and severe disease of 1.75–2.10 and 2.62–3.93, respectively; as with the seropositive group calculations, the CIs were narrow and crossed 1 in only one analytic method.

Data pooled from CYD14 and 15 were used to assess vaccine efficacy by serostatus between study months 0 and 25. In 9–16-year-olds, vaccine efficacy against symptomatic dengue in seropositive individuals ranged from 74% to 77% depending on the analytic method, and CIs were tight with a range of 64–84%. The efficacy in seronegatives was less with large CIs and two of the three methods resulting in crossing of 0 (18–45% efficacy range, 18–63% CI range). In the 2–8 year age range, efficacy in seropositives was less, ranging from 57% to 70% with a CI range of 31–82%. Younger seronegatives also had lower efficacy, ranging from 8% to 28% with all CIs crossing 0. Combining age ranges (2–16 years) maintained the positive vaccine effect in the seropositive group (71–75% with CI range 59–82%). Seronegatives continued to have lower efficacy (15–40%) with two of three CIs crossing 0 (CI range15-58%).

DENV type-specific efficacy between study months 0–25 in seropositive 9–16-year-olds (pooled data CYD 14 and 15) demonstrated superior efficacy against DENV-4 (89.3% [79.8–94.4]) and DENV-3 (80.0% [67.3–87.7]) and lower and similar efficacy against DENV-1 (67.4% [45.9–80.4]) and DENV-2 (67.3 [46.7–79.9]).

The Sponsor evaluated the clinical phenotypes of all hospitalizations occurring in seronegative subjects from study month 13 to the end of the follow-up period (month 60 to month 72) using pooled data from CYD23 (57), 14, and 15. Outcome data were assessed in age ranges 2–16, 9–16, and 2–8 years. There were no substantial differences between vaccine and control groups across any of the age ranges as it related to median duration of symptoms (7.5–8 days), median duration of fever (5 days), median duration of hospitalization (4–5 days), occurrence of any hemorrhage (39.3–45% of group), or any visceral manifestation of disease (0–5% of group). There was, however, measurable differences in the occurrence of plasma leakage (any clinical signs, hematocrit increase >/ = 20%) and thrombocytopenia (</ = $50x10^9$/liter or </ = $100x10^9$/liter). The risk ratios favored control for all measurements of plasma leakage; however, the number of cases with plasma leakage was small in some categories in some age groups making the CIs quite large and crossing 1. Risk ratios also favored control related to the occurrence of thrombocytopenia, especially for counts </ = $50x10^9$/liter; CIs were reasonably narrow in all groups and did not cross 1 except in the 9–16 age range. There were very few (N = 6) cases of shock and they were all in the vaccine groups (2–16 years, N = 3; 2–8 years, N = 3).[70]

Risk of hospitalization assessed as a function of infecting DENV type was evaluated following at least one dose of vaccine in seropositive 9–16-year-olds using pooled data from CYD23 (57), 14, and 15 measured over a 5–6-year period. All hazard ratios favored Dengvaxia® with DENV-4 (0.07 [0.01–0.38]) having the most favorable ratio followed by DENV-2 (0.18 [0.09–0.34]), DENV-1 (0.22 [0.11–0.45]), and DENV-3 (0.38 [0.17–0.82]).

Risk of hospitalization assessed as a function of time from vaccination (seropositive, 9–16-year-olds, pooled data) indicated the lowest risk in the first 2 years of the study (0.108 [0.054–0.215]), relative stabilization during years 3 (0.263 [0.113–0.612]) and 4 (0.219 [0.102–0.486]), and a decrement in year 5 and partial year 6 (0.456 [0.215–0.965]). The data in seropositive 2–8-year-olds (pooled CYD23 (57) and 14) were less compelling for a vaccine benefit, with hazard ratios for hospitalized and severe dengue in 2–5-year-olds of 0.73 (0.41–1.31) and 1.03 (0.33–3.23), respectively. In 6–8-year-olds, the hazard ratio for hospitalization was robust (0.40 [0.24–0.68]), but not for severe dengue (0.34 [0.11–1.09]). Cumulative incidence curves for hospitalization and severe dengue risk in seronegative 9–16-year-olds demonstrated a crossing of the vaccine and control curves at approximately study month 30, reflecting the increased risk of both outcomes in the vaccine group.

Finally, to determine if Dengvaxia® protected against subclinical infection, phase 3 trial data from the first 25 months (active surveillance) was analyzed, with asymptomatic infection defined by four-fold rise in annual neutralizing antibody titer; 219/2,485 (8.8%) in the Dengvaxia® group vs 157/1,184 (13.3%) in the placebo group seroconverted between months 13 and 25, yielding vaccine efficacy against asymptomatic infections of 33.5% (95%CI, 17.9–46.1) during this period.[93]

In summary, there were clear trends toward superior efficacy and a beneficial vaccine effect in seropositive versus seronegative vaccine recipients. Furthermore, there appeared to be a trend of superior efficacy and beneficial vaccine effect in older children compared to the younger age groups. Protective effects were noted against any dengue caused by all DENV types as well as severe and hospitalized dengue. In contrast, there was a clear safety signal in seronegative vaccine recipients especially in the younger age groups. Hospitalization and severe disease occurred with increased frequency in seronegative vaccine recipients compared to control starting at approximately 18 months following the last dose of vaccine (or study month 30). These data moved the Sponsor to seek an indication in children 9 years of age or greater and in those seropositive to dengue at baseline or with a confirmed history of past DENV infection.

Understanding Dengvaxia® performance

Dengvaxia's® development pathway was guided by decades of lessons learned from the development of live virus dengue vaccine candidates, pre-clinical and early clinical data, demonstrating a promising safety and immunogenicity profile, and the guidance of groups such as the WHO.[23,78-80,94-101] There was confidence the prevention of viremia or RNAemia in NHPs challenged with wild-type viruses would be predictive of the potential for human clinical benefit. It was also anticipated anti-DENV neutralizing antibodies would emerge as a correlate or surrogate of protection, supporting the plausibility that a neutralizing antibody response would protect against dengue. Dengvaxia's® failure to meet the primary efficacy end-point in CYD23 was unexpected and called into question many commonly held beliefs in the dengue vaccine research community. The vaccine's variable performance across different ages and serostatus as well as a clear safety signal in seronegative recipients, i.e., that Dengvaxia® enhanced subsequent disease in some seronegative individuals, raised a number of questions and concepts applicable to all dengue vaccine candidates.

Immunodominance

One of the challenges in developing dengue vaccines is the requirement to make a safe and effective vaccine against each DENV type, and then to successfully combine them into a single formulation.[102-104] With replicating DENV vaccine platforms, immunodominance and interference are theoretical risks which may result in having a single DENV type dominating the antigen presentation process and thereby skew immune responses and associated protective capability. There is evidence these concepts may be at play with Dengvaxia®. Following tetravalent vaccination with CYD in cynomolgus macaques, DENV-4, and to a lesser extent, DENV-1, were dominant in terms of neutralizing antibody response, and DENV-4 vaccine viremia was detected.[96] In a phase 2 trial, subjects were vaccinated on Day 0 and Day 105 with four regimens consisting of bivalent and/or tetravalent formulations: (1) CYD-1, 3 followed by CYD-2, 4; (2) CYD-1, 3, 4 + VDV2 x 2; (3) Dengvaxia® x 2; and (4) JE vaccine followed by Dengvaxia®. CYD-4 RNAemia was most frequent after tetravalent vaccination. In group one, CYD-3 RNAemia was most frequent after the first bivalent injection (CYD-1,3), with the highest immune response being against DENV-3. The second bivalent injection (CYD-2,4) elicited only low immune response against DENV-2 and −4.[58] In another phase 2 trial of dengue naïve adults in Australia, CYD RNAemia was detected in 66/95 (69.5%) subjects who received Dengvaxia®, most within 6–14 days after the first dose. The most commonly detected vaccine type was DENV-4 (45/95 [47.4%]), followed by DENV-3 (13/95 [13.7%]), and DENV-1 (8/95 [8.4%]); DENV-2 was not detected.[105]

Immunogenicity data from five Dengvaxia® phase 2 trials were analyzed to evaluate immune response after each of the three doses. Neutralizing antibody titers were similar for all types following the third dose, but DENV-4 was immunodominant after the first dose.[106] Depletion of cross-reactive dengue antibodies followed by a flow cytometry-based neutralization assay was performed on a small number of phase 2 subjects with baseline dengue seropositivity (7 subjects from Brazil) and seronegativity (11 subjects from Australia). The intent was to evaluate DENV type-specific versus cross-reactive neutralizing antibodies following Dengvaxia® administration. In baseline seronegative individuals, Dengvaxia® induced DENV-4 type-specific neutralizing antibodies but only low levels of DENV-1, −2, or −3 type-specific antibodies. In baseline seropositive individuals, Dengvaxia® induced mainly cross-reactive antibodies, and maintained or boosted pre-existing DENV type-specific neutralizing antibodies.[107]

Immunogenicity as a poor predictor of efficacy

The association of quantitative neutralizing antibody responses after Dengvaxia® with protection in phase 2b and 3 efficacy trials was evaluated.[108] Neutralizing antibodies measured 1 month after the third vaccine dose in the immunogenicity subsets from CYD 14 and 15 were analyzed to determine their association with protection against symptomatic dengue thru 25 months after the first dose. Vaccinees with higher month 13 titers to a particular DENV type had a lower risk of symptomatic dengue from that type (hazard ratio of 0.19–0.43 per 10-fold increase in titer). Vaccinees with higher month 13 mean titers to four DENV types had higher efficacy against symptomatic dengue from any type. However, the lowest titers did not correspond to zero efficacy, suggesting a role for other factors in protection, and demonstrating that neutralizing antibody titers may only be a crude quantitative indicator of clinically relevant immune responses.

To evaluate the role of conformational epitopes, antigenic properties of DENV-1–4 were characterized using highly neutralizing human monoclonal antibodies (hmAbs) binding quaternary epitopes. Binding of DENV-1 (1F4), DENV-2 (2D22), or DENV-3 (5J7) type-specific hmAbs, and DENV-1–4 cross-reactive hmAb (1C19); demonstrated that Dengvaxia® had high functional affinity with these hmAbs. These findings suggest Dengvaxia® can elicit at least some antibody responses with DENV type-specific specificity similar to what is observed following a natural infection. However, the number of tested conformational epitopes was very few, limiting broader conclusions about antibody specificities.[109]

Cellular immune responses following vaccination

Cellular immune responses to natural DENV infections are known to participate in both pathologic and protective immune responses. The majority of CD4+ and CD8 + T cell epitopes are located on the DENV non-structural proteins; the CYD vaccine does not possess these proteins.[110-116] In the absence of non-structural proteins, it is reasonable to postulate Dengvaxia® would not elicit robust cellular immune responses and this may contribute to the absence of protection or reduce the durability and duration of protection remote from vaccination. In fact, Dengvaxia® elicited mostly YF 17D NS3-specific CD8+ responses and DENV type-specific CD4+ responses. Of interest,

pre-existing DENV NS3-specific CD8+ responses could be recalled by Dengvaxia® administration. Dengvaxia® administration favored responses with IFN-gamma secreting T cells over TNF-alpha secreting T cells, suggesting a Th1 over Th2 response.[65,117]

Theories explaining Dengvaxia's® variable safety and efficacy signals

Does Dengvaxia® mimic a first infection

One hypothesis explaining Dengvaxia's® safety issues in seronegative recipients is that vaccination mimics a primary infection, setting up the recipient for the higher risk of clinically apparent and/or severe disease when he or she experiences a subsequent sequential infection which, in this case, would be the recipient's first natural infection.[118] Inherent in this hypothesis is the assumption that Dengvaxia® elicits homotypic immune responses from ≤ three serotypes; in fact, given DENV-4's likely immunodominance, CYD-TDV may elicit homotypic immune response mainly for just DENV-4. In this scenario, vaccine recipients would benefit from durable DENV-4 homotypic protection, but only a short period of cross-protective immunity against other serotypes. This could account for the relatively positive efficacy signal in study years 0–2 of the efficacy trials, and the absence of a safety signal. Once heterotypic immunity begins to wane, vaccine recipients would begin to experience natural infections, possibly accounting for the safety signal appearing only in year 3, and the decline in risk during years 4 and beyond.[119]

However, the analogy of mimicking a first natural infection is potentially problematic for a number of reasons. First and foremost, there are considerable differences between a wild-type DENV delivered by a mosquito versus needle administration of a vaccine. A probing mosquito delivers a single DENV type, complete viral genome (structural and non-structural proteins) into a superficial anatomic space along with a variety of mosquito salivary proteins.[120-122] A second, sequential infection would then be experienced months to years later. In contrast, Dengvaxia® is delivered by a needle into the subcutaneous tissue, contains only the prM and E genes from each of the four DENV types, and is administered three times over a single year. The differences in virus/antigen make-up and method of vaccine delivery all have the potential to impact antigen processing and resulting immune responses, thereby limiting the comparison.

Does Dengvaxia® fail to produce a cellular immune response

Another potential explanation for Dengvaxia's® variable performance is its failure to elicit potent and broad cellular immune responses. High, post-vaccination antibody titers in the absence of a cellular response could account for near-term protection, but as this antibody response wanes, cellular immune responses are likely required for robust immunologic memory and an overall protective immune profile. Depending on the vaccine construct, a frequent schedule of booster doses may be required to overcome this challenge.

Does Dengvaxia® fail to target relevant DENV epitopes

Relevant epitopes for protection from natural infection with a wild-type virus (e.g., conformational epitopes) may differ from the DENV components in Dengvaxia®.

DENGVAXIA'S® waning immunogenicity

Blood was collected from a small number of volunteers 5 years after vaccination as part of a phase 2 trial in Singapore. In the original trial, 57–84% of volunteers had a neutralizing antibody response (titer ≥10) to all DENV types when measured 28 days after a third dose of vaccine. Five years after the first dose of vaccine, 21/23 had no antibodies measured by ELISA, and DENV-specific memory B cells were low. The *in vivo* ability of plasma antibodies to impact viremia after DENV-2 challenge in a mouse model was poor with only 2 out of 23 samples demonstrating reduced viremia.[123]

Somewhat conflicting data were published by the Sponsor in a review of 10 phase 2 and six phase 3 trials conducted in dengue endemic and non-endemic regions. Neutralizing antibodies from available samples were determined at baseline, 28 days after the third dose of vaccine, and annually up to 4 years after the third dose. Dengvaxia® elicited neutralizing antibodies against each DENV type with increasing GMTs following each dose. In CYD 14 and 15, GMTs decreased initially during the first 2 years post-dose 3, but appeared to stabilize or slightly increase again in year three. When measured out to year four, GMTs persisted at 1.2–3.2-fold higher than baseline. Numerous factors impacted antibody titer and kinetics of decline to include age, dengue serostatus at baseline, and region.[124]

Pre-existing immunity to non-DENV flaviviruses

Fourteen subjects previously vaccinated against yellow fever received a single dose CYD-2 with all subjects developing an antibody response to all DENV types which persisted for at least 1 year. The yellow fever naïve subjects had low response rates to DENV-1, 3 and 4.[52] In another phase 1 trial, a regimen of TDV-TDV-TDV elicited seropositivity against each of DENVs between 77% and 92% while YF-TDV-TDV elicited seropositivity between 85% and 94%.[55] Dengvaxia® administered 1 year after live attenuated dengue vaccine (VDV) types 1 or 2, or YF vaccine demonstrated that 67% (10/15) of VDV-primed subjects seroconverted to all DENV types within 28 days compared with only 25% (2/8) of YF-primed and 0% (0/12) of naïve subjects.[57] In a study of JEV priming, JE vaccine was administered on days −14, −7 and 0 followed by Dengvaxia® on day 105. Immune responses after Dengvaxia® injection in JE-primed individuals were higher than after one Dengvaxia® injection in dengue naïve subjects.[58]

Does Dengvaxia® miss relevant DENV genotypes

One study evaluated the genetic epidemiology of DENVs collected from dengue cases in CYD14 and 15 allowing DENV genotype-specific estimates of vaccine efficacy. Envelope gene sequences (n = 661) from 11 DENV genotypes in 10 endemic countries demonstrated high amino acid identity between the CYD strains and wild-type viruses. Similarities included epitope sites targeted by neutralizing human monoclonal antibodies. Post-hoc analysis of all CYD14 and 15 trial participants revealed a statistically significant genotype-level efficacy association within DENV-4 while a subgroup analysis of efficacy in 9–16-year-olds appeared more balanced within each DENV type.[125]

Sieve analyses were conducted to evaluate if Dengvaxia® efficacy varied with amino acid sequence features of the DENVs isolated from infected subjects. DENV prM and E amino acid sequences from cases were aligned with the vaccine insert sequences, and extensions of the proportional hazards model were applied to assess variation in efficacy with amino acid mismatch proportion distances from vaccine strains, individual amino acid residues, and phylogenetic genotypes. In CYD14, efficacy against any DENV type decreased significantly with increasing amino acid distance from the vaccine, whereas in CYD15, efficacy was not impacted by amino acid distance. In DENV type-specific analyses, there was a decrement in efficacy against DENV-4 with increasing amino acid distance from the corresponding vaccine insert, but these observations were limited to 2–-8-year-olds. Finally, there was greater estimated efficacy against DENV types and genotypes when the circulating DENVs had shorter amino acid sequence distances from the vaccine.[126]

Immunization implementation

World health organization position evolution

The WHO has issued two position papers on Dengvaxia®, one based on the data used for initial licensure and the second addressing modifications to the initial indication based on the observed safety signal in seronegative vaccine recipients in all ages studied. The first position was issued in July 2016 and contained the following observations and recommendations:

(1) CYD-TDV should only be introduced in geographic settings where epidemiological data indicate a high burden of disease (seroprevalence 70% or greater in the age group targeted for vaccination);
(2) The vaccine is not recommended when seroprevalence is below 50% in the age group targeted for vaccination;
(3) Use of CYD-TDV in populations where seroprevalence is low in the age group considered for vaccination is not recommended;
(4) If CYD-TDV is introduced, it should be administered as a 3-dose series given on a 0/6/12-month schedule;
(5) Because of the association of CYD-TDV with increased risk of hospitalized and severe dengue illness in the 2–5-year age group, CYD-TDV is not recommended for use in children under 9 years of age;
(6) CYD-TDV has not been studied as an intervention for dengue outbreak control and is not expected to have a significant impact on the course of the ongoing outbreak; and
(7) Long-term monitoring for severe dengue, in particular in seronegative vaccinated subjects, should be done in selected areas.

Based on WHO's recommendation #7 and the increasing evidence of a detrimental effect of vaccination in seronegative individuals, the Sponsor embarked on exploring the effect of age and serostatus on vaccine efficacy and long-term safety (described earlier). Based on the data, the Sponsor requested a change in the vaccine's label:

> Based on up to six years of clinical data … For those not previously infected by dengue virus, however, the analysis found that in the longer term, more cases of severe disease could occur following vaccination upon a subsequent dengue infection … Based on the new analysis, Sanofi will propose that national regulatory agencies update the prescribing information … For individuals who have not been previously infected by dengue virus, vaccination should not be recommended. November 29, 2017

(https://mediaroom.sanofi.com/en/press-releases/2017/sanofi-updates-information-on-dengue-vaccine/, accessed 7 MAY 2019)

The WHO revised their position based on this new information in September 2018:

(1) CYD-TDV has been shown in clinical trials to be efficacious and safe in seropositive individuals, but carries an increased risk of severe dengue in those who experience their first natural dengue infection after vaccination (seronegative individuals);
(2) Only people with evidence of a past dengue infection should be vaccinated based on an antibody test, or on a documented laboratory confirmed dengue infection in the past. If pre-vaccination screening is not feasible, vaccination without individual screening could be considered in areas with recent documentation of seroprevalence rates of at least 80% by age 9 years;
(3) Screening tests would need to be highly specific to avoid vaccinating truly seronegative persons and to have high sensitivity to ensure that a high proportion of seropositive persons are vaccinated;
(4) Decisions about implementing a pre-vaccination screening strategy with the currently available tests will require careful assessment at the country level, including consideration of the sensitivity and specificity of available tests;
(5) The vaccine should be used within the indicated age range, which in most countries is 9–45 years. The age group to target for vaccination depends on the dengue transmission intensity in a given country;
(6) In travelers who have already had a documented dengue illness or are seropositive, vaccination before travel to high dengue transmission settings could be considered; and

(7) There is an urgent need for the development of highly specific and sensitive rapid diagnostic tests (RDTs) for determination of dengue serostatus.

155th vaccines and related biological products advisory committee (VRBPAC) – 7 MAR 2019

The VRBPAC evaluated Dengvaxia® in March 2019. The Sponsor's presentations focused on the unmet medical need for a dengue vaccine in the U.S., highlighting the potential for high dengue rates and death in Puerto Rico. The vaccine's safety and efficacy were reviewed with a not so surprising focus on the safety signal in seronegative vaccine recipients. The requirement and availability of a widely accessible diagnostic with high sensitivity and specificity was discussed at length. There was broad support for the need for a U.S. FDA cleared rapid diagnostic assay to accurately identify seropositive individuals. A number of committee members had issue with extrapolating immunogenicity (non-efficacy) data to the populations above 16 years of age.

The U.S. FDA requested the VRBPAC consider two questions:

(1) Are the available data adequate to support the effectiveness of Dengvaxia® for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in persons 9 through 45 years of age with laboratory-confirmed previous dengue infection and living in endemic areas?
(2) Are the available data adequate to support the safety of Dengvaxia® when administered to persons 9 through 45 years of age with laboratory-confirmed previous dengue infection and living in endemic areas?

Voting on these questions was almost evenly split with question 1 votes tallying at 6 Yes, 7 No, and 1 Abstain and question 2 voting at 7 Yes, 7 No, and 0 Abstain.

The FDA then asked, in real-time, the VRBPAC to consider two additional questions:

(3) Are the available data adequate to support the effectiveness of Dengvaxia® for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in persons 9 to <17 years of age with laboratory-confirmed previous dengue infection and living in endemic areas?
(4) Are the available data adequate to support the safety of Dengvaxia® when administered to persons 9 through <17 years of age with laboratory-confirmed previous dengue infection and living in endemic areas?

There was increased committee consensus on questions 3 and 4 with voting of 13 Yes, 1 No, and 0 Abstain for question 3 and 10 Yes, 4 No, and 0 Abstain for question 4.

On 1 May 2019, U.S. FDA announced the approval of Dengvaxia®, "the first vaccine approved for the prevention of dengue disease caused by all dengue virus serotypes (1, 2, 3 and 4) in people ages 9 through 16 who have laboratory-confirmed previous dengue infection and who live in endemic areas."(https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-dengue-disease-endemic-regions; accessed 7 May 2019).

Dengvaxia® roll-out and fall-out

Of the 2.9 million doses of vaccine which have been distributed worldwide, approximately 2.3 million doses have been used during campaigns in the Philippines and Brazil. In the Philippines >830,000 children received one dose, >415,000 two doses, and >365,000 all three doses. In Brazil, the distribution was 300,000 dose 1, 225,000 dose 2, and 146,000 dose 3. A surveillance system was in place by the Philippine Department of Health at the start of vaccinations, with the collection of all AEs following immunization (AEFI). AE causality was made by an AEFI committee. Surveillance for AEs among Dengvaxia® recipients increased following the release of the safety signal in seronegative individuals above 9 years of age. Nevertheless, the robustness of these systems to detect AEFIs, and in particular, to evaluate dengue cases related to vaccination, was unclear. In Brazil, a public vaccination program was conducted in Parana state, and the existing surveillance system was purportedly enhanced in preparation for the campaign.

From initial marketing of the vaccine until 14 September 2018, 51 fatalities were reported in the Sanofi Pharmacovigilance database. The majority of fatal cases occurred in children (9–13 years) in the Philippines and 15 of the 51 were dengue cases. The WHO Global Advisory Committee on Vaccine Safety reviewed 14 deaths and were unable to make a causality determination, " … *in the absence of criteria for distinguishing vaccine failure from vaccine-related immune enhancement, individual cases cannot be attributed to one or the other. As a result, such cases should be classified as indeterminate, irrespective of the time since vaccination.*"[11]

It is beyond the scope of this manuscript to dissect the political, legal, and social elements associated with Dengvaxia's® development, launch, and subsequent challenges. That being said, the erosion of vaccine confidence in the Philippines post-Dengvaxia®, criminal indictments of Philippine medical professionals and Sanofi officials, and the revocation of Dengvaxia's® license in the Philippines are highly concerning occurrences. The scientific, ethical, legal, and regulatory turbulence resulting from this experience has the potential to impact the development and deployment of future dengue vaccines and potentially other technologies designed to prevent or treat dengue.

Lessons learned

There have been numerous lessons learned from the Dengvaxia® experience and dengue vaccine development efforts in general:

(1) A more in-depth understanding of the induction, kinetics, and contributions to safety and protection

of long-term homotypic, transient heterotypic, and long-term heterotypic immune responses is required, which will, in turn, require better ways to measure them;

(2) Multivalent replicating vaccines are at theoretical risk of experiencing immunodominance and immune interference in the recipient, likely necessitating a more iterative development approach to evaluate individual infectivity and immunogenicity (example – exploring monovalent dengue vaccines in separate clinical studies prior to combination);

(3) Since clinically relevant immune responses can change over time after natural infection or vaccination, the timing of efficacy measurements will need to be taken into account when considering vaccine efficacy and risk;

(4) Surveillance systems applied to vaccine efficacy trials should be designed to capture clinical end-points of interest for the period of time required to make a maximally informed decision about the vaccine's potential for clinical benefit (i.e., how many dengue seasons?);

(5) Exploring immunogenicity and efficacy as a function of vaccine viral strains and contemporary circulating DENV types and genotypes should be considered by Sponsors, especially those using vaccine strains collected many years prior;

(6) Understanding the impact of age, baseline dengue and non-dengue flavivirus serostatus, infecting serotype, and time from vaccination on immunogenicity, efficacy, and safety should be a focus of Sponsors;

(7) Expanding and standardizing methods to complete quantitative and qualitative measures of humoral immune responses are required to leverage an understanding of protective and deleterious responses and what constitutes each (i.e., target epitopes);

(8) Exploring, in a prospective manner, immune correlates or surrogates of protection and risk should be a Sponsor priority, and will likely require collecting baseline blood samples on all trial participants, lengthening the duration of active surveillance, and having secondary efficacy endpoints assessing various time points remote from the vaccination; and

(9) Use of experimental human infection models should be considered to assist with early development decisions (i.e., antigen selection, dose, and schedule), gaining an early understanding of a vaccine candidate's potential for clinical benefit prior to large clinical endpoint studies, and potentially adding to a data package supporting pursuit of a specific indication (example – fillings gaps in knowledge from field efficacy studies).

Conclusion

The development and licensure of Dengvaxia® spanned more than 20 years and cost more than 1.5 billion U.S. dollars. The breadth and depth of the pre-clinical and clinical development pathways addressed a variety of real and theoretical risks inherent to all vaccine development efforts and some which were dengue-specific. Although two large phase 3 efficacy trials met primary efficacy endpoints, long-term follow-up revealed a very concerning safety signal in seronegative vaccine recipients. Additional data generation and analyses confirmed the signal, resulting in a modification of the requested indication from the Sponsor, WHO recommendations for use, and approvals from regulatory agencies. The current storm of political, legal, and community fall-out continues without a clear understanding of the final outcome.

Author contributions

IKY and SJT contributed equally to all aspects of this article.

Disclosure of potential conflicts of interest

IKY's institution has received unrestricted grants from Sanofi Pasteur. SJT and IKY consult for a number of companies developing countermeasures against dengue, including Sanofi Pasteur, and they are financially compensated for their time.

ORCID

Stephen J. Thomas http://orcid.org/0000-0002-8368-7682

References

1. Gubler DJ. Dengue, urbanization and globalization: The Unholy Trinity of the 21(st) century. Trop Med Health. 2011;39(4 Suppl):3–11. PubMed PMID: 22500131; PubMed Central PMCID: PMCPMC3317603. doi:10.2149/tmh.2011-S05.
2. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. Nat Rev Dis Primers. 2016;2:16055. PubMed PMID: 27534439. doi:10.1038/nrdp.2016.55.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, et al. The global distribution and burden of dengue. Nature. 2013;496 (7446):504–07. PubMed PMID: 23563266; PubMed Central PMCID: PMCPMC3651993. doi:10.1038/nature12060.
4. Salje H, Cummings DAT, Rodriguez-Barraquer I, Katzelnick LC, Lessler J, Klungthong C, Thaisomboonsuk B, Nisalak A, Weg A, Ellison D, et al. Reconstruction of antibody dynamics and infection histories to evaluate dengue risk. Nature. 2018;557 (7707):719–23. 10.1038/s41586-018-0157-4. PubMed PMID: 29795354. doi:10.1038/s41586-018-0157-4.
5. Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. Proc Natl Acad Sci U S A. 2016;113(3):728–33. PubMed PMID: 26729879; PubMed Central PMCID: PMCPMC4725482. doi:10.1073/pnas.1522136113.
6. Anderson KB, Gibbons RV, Cummings DA, Nisalak A, Green S, Libraty DH, Jarman RG, Srikiatkhachorn A, Mammen MP, Darunee B, et al. A shorter time interval between first and second dengue infections is associated with protection from clinical illness in a school-based cohort in Thailand. J Infect Dis. 2014;209(3):360–68. PubMed PMID: 23964110; PubMed Central PMCID: PMCPMC3883164. doi:10.1093/infdis/jit436.
7. Montoya M, Gresh L, Mercado JC, Williams KL, Vargas MJ, Gutierrez G, Kuan G, Gordon A, Balmaseda A, Harris E, et al. Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year. PLoS Negl Trop Dis. 2013;7(8):e2357. PubMed

PMID: 23951377; PubMed Central PMCID: PMCPMC3738476. doi:10.1371/journal.pntd.0002357.
8. Halstead SB. Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med. 1970;42:350–62.
9. Guzman MG, Harris E. Dengue. Lancet. 2015;385(9966):453–65. PubMed PMID: 25230594. doi:10.1016/S0140-6736(14)60572-9.
10. Sabin AB, Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice. Science. 1945;101 (2634):640–42. PubMed PMID: 17844088. doi:10.1126/science.101.2634.640.
11. Dengue vaccine: WHO position paper, September 2018 - Recommendations. Vaccine. 2018. PubMed PMID: 30424888. doi:10.1016/j.vaccine.2018.09.063
12. Chambers TJ, Nestorowicz A, Mason PW, Rice CM. Yellow fever/Japanese encephalitis chimeric viruses: construction and biological properties. J Virol. 1999;73(4):3095–101. PubMed PMID: 10074160; PubMed Central PMCID: PMCPMC104070.
13. Guirakhoo F, Weltzin R, Chambers TJ, Zhang ZX, Soike K, Ratterree M, Arroyo J, Georgakopoulos K, Catalan J, Monath TP. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. J Virol. 2000;74(12):5477–85. PubMed PMID: 10823852; PubMed Central PMCID: PMCPMC112032. doi:10.1128/jvi.74.12.5477-5485.2000.
14. Guirakhoo F, Arroyo J, Pugachev KV, Miller C, Zhang ZX, Weltzin R, Georgakopoulos K, Catalan J, Ocran S, Soike K, et al. Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. J Virol. 2001;75(16):7290–304. PubMed PMID: 11462001; PubMed Central PMCID: PMCPMC114964. doi:10.1128/JVI.75.16.7290-7304.2001.
15. Mantel N, Girerd Y, Geny C, Bernard I, Pontvianne J, Lang J, Barban V. Genetic stability of a dengue vaccine based on chimeric yellow fever/dengue viruses. Vaccine. 2011;29(38):6629–35. PubMed PMID: 21745519. doi:10.1016/j.vaccine.2011.06.101.
16. Zhang Y, Corver J, Chipman PR, Zhang W, Pletnev SV, Sedlak D, Baker TS, Strauss JH, Kuhn RJ, Rossmann MG. Structures of immature flavivirus particles. Embo J. 2003;22(11):2604–13. PubMed PMID: 12773377; PubMed Central PMCID: PMCPMC156766. doi:10.1093/emboj/cdg270.
17. Kuhn RJ, Zhang W, Rossmann MG, Pletnev SV, Corver J, Lenches E, Jones CT, Mukhopadhyay S, Chipman PR, Strauss EG, et al. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell. 2002;108 (5):717–25. PubMed PMID: 11893341; PubMed Central PMCID: PMCPMC4152842. doi:10.1016/s0092-8674(02)00660-8.
18. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine. 2011;29(42):7229–41. PubMed PMID: 21745521. doi:10.1016/j.vaccine.2011.06.094.
19. Guy B, Guirakhoo F, Barban V, Higgs S, Monath TP, Lang J. Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. Vaccine. 2010;28(3):632–49. PubMed PMID: 19808029. doi:10.1016/j.vaccine.2009.09.098.
20. Dubayle J, Vialle S, Schneider D, Pontvianne J, Mantel N, Adam O, Guy B, Talaga P. Site-specific characterization of envelope protein N-glycosylation on Sanofi Pasteur's tetravalent CYD dengue vaccine. Vaccine. 2015;33(11):1360–68. PubMed PMID: 25660649. doi:10.1016/j.vaccine.2015.01.047.
21. Deauvieau F, Sanchez V, Balas C, Kennel A, DEM A, Lang J, Guy B. Innate immune responses in human dendritic cells upon infection by chimeric yellow-fever dengue vaccine serotypes 1-4. Am J Trop Med Hyg. 2007;76(1):144–54. PubMed PMID: 17255244.
22. Higgs S, Vanlandingham DL, Klingler KA, McElroy KL, McGee CE, Harrington L, Lang J, Monath TP, Guirakhoo F. Growth characteristics of ChimeriVax-Den vaccine viruses in Aedes aegypti and Aedes albopictus from Thailand. Am J Trop Med Hyg. 2006;75(5):986–93. PubMed PMID: 17124001.
23. Edelman R, Hombach J. "Guidelines for the clinical evaluation of dengue vaccines in endemic areas": summary of a World Health Organization Technical Consultation. Vaccine. 2008;26 (33):4113–19. PubMed PMID: 18597906. doi:10.1016/j.vaccine.2008.05.058.
24. Pugachev KV, Guirakhoo F, Ocran SW, Mitchell F, Parsons M, Penal C, Girakhoo S, Pougatcheva SO, Arroyo J, Trent DW, et al. High fidelity of yellow fever virus RNA polymerase. J Virol. 2004;78(2):1032–38. PubMed PMID: 14694136; PubMed Central PMCID: PMCPMC368746. doi:10.1128/jvi.78.2.1032-1038.2004.
25. Barban V, Girerd Y, Aguirre M, Gulia S, Pétiard F, Riou P, Barrere B, Lang J. High stability of yellow fever 17D-204 vaccine: a 12-year restrospective analysis of large-scale production. Vaccine. 2007;25(15):2941–50. PubMed PMID: 16914238. doi:10.1016/j.vaccine.2006.06.082.
26. McGee CE, Tsetsarkin K, Vanlandingham DL, McElroy KL, Lang J, Guy B, Decelle T, Higgs S. Substitution of wild-type yellow fever Asibi sequences for 17D vaccine sequences in ChimeriVax-dengue 4 does not enhance infection of Aedes aegypti mosquitoes. J Infect Dis. 2008;197(5):686–92. PubMed PMID: 18266608; PubMed Central PMCID: PMCPMC2597369. doi:10.1086/527328.
27. McGee CE, Lewis MG, Claire MS, Wagner W, Lang J, Guy B, Tsetsarkin K, Higgs S, Decelle T. Recombinant chimeric virus with wild-type dengue 4 virus premembrane and envelope and virulent yellow fever virus Asibi backbone sequences is dramatically attenuated in nonhuman primates. J Infect Dis. 2008;197 (5):693–97. PubMed PMID: 18266603. doi:10.1086/527329.
28. Johnson BW, Chambers TV, Crabtree MB, Guirakhoo F, Monath TP, Miller BR. Analysis of the replication kinetics of the ChimeriVax-DEN 1, 2, 3, 4 tetravalent virus mixture in Aedes aegypti by real-time reverse transcriptase-polymerase chain reaction. Am J Trop Med Hyg. 2004;70(1):89–97. PubMed PMID: 14971704. doi:10.4269/ajtmh.2004.70.89.
29. Monath TP, Myers GA, Beck RA, Knauber M, Scappaticci K, Pullano T, Archambault WT, Catalan J, Miller C, Zhang Z-X, et al. Safety testing for neurovirulence of novel live, attenuated flavivirus vaccines: infant mice provide an accurate surrogate for the test in monkeys. Biologicals. 2005;33(3):131–44. PubMed PMID: 15975826. doi:10.1016/j.biologicals.2005.03.009.
30. Ravel G, Mantel N, Silvano J, Rogue A, Guy B, Jackson N, Burdin N. Biodistribution and safety of a live attenuated tetravalent dengue vaccine in the cynomolgus monkey. Vaccine. 2017;35(43):5918–23. PubMed PMID: 28882438. doi:10.1016/j.vaccine.2017.08.071.
31. Brandler S, Brown N, Ermak TH, Mitchell F, Parsons M, Zhang Z, Lang J, Monath TP, Guirakhoo F. Replication of chimeric yellow fever virus-dengue serotype 1-4 virus vaccine strains in dendritic and hepatic cells. Am J Trop Med Hyg. 2005;72(1):74–81. PubMed PMID: 15728870.
32. Balas C, Kennel A, Deauvieau F, Sodoyer R, Arnaud-Barbe N, Lang J, Guy B. Different innate signatures induced in human monocyte-derived dendritic cells by wild-type dengue 3 virus, attenuated but reactogenic dengue 3 vaccine virus, or attenuated nonreactogenic dengue 1-4 vaccine virus strains. J Infect Dis. 2011;203(1):103–08. PubMed PMID: 21148502; PubMed Central PMCID: PMCPMC3086443. doi:10.1093/infdis/jiq022.
33. Guirakhoo F, Pugachev K, Arroyo J, Miller C, Zhang ZX, Weltzin R, Georgakopoulos K, Catalan J, Ocran S, Draper K, et al. Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine: genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. Virology. 2002;298(1):146–59. PubMed PMID: 12093182. doi:10.1006/viro.2002.1462.
34. Guirakhoo F, Pugachev K, Zhang Z, Myers G, Levenbook I, Draper K, Lang J, Ocran S, Mitchell F, Parsons M, et al. Safety and efficacy of chimeric yellow Fever-dengue virus tetravalent

vaccine formulations in nonhuman primates. J Virol. 2004;78 (9):4761–75. PubMed PMID: 15078958; PubMed Central PMCID: PMCPMC387722. doi:10.1128/jvi.78.9.4761-4775.2004.
35. Guy B, Barban V, Mantel N, Aguirre M, Gulia S, Pontvianne J, Jourdier T-M, Ramirez L, Gregoire V, Charnay C, et al. Evaluation of interferences between dengue vaccine serotypes in a monkey model. Am J Trop Med Hyg. 2009;80(2):302–11. PubMed PMID: 19190230.
36. Barban V, Munoz-Jordan JL, Santiago GA, Mantel N, Girerd Y, Gulia S, Claude J-B, Lang J. Broad neutralization of wild-type dengue virus isolates following immunization in monkeys with a tetravalent dengue vaccine based on chimeric yellow fever 17D/dengue viruses. Virology. 2012;429(2):91–98. PubMed PMID: 22542002. doi:10.1016/j.virol.2012.03.007.
37. Barban V, Mantel N, De Montfort A, Pagnon A, Pradezynski F, Lang J, Boudet F. Improvement of the dengue virus (DENV) nonhuman primate model via a reverse translational approach based on dengue vaccine clinical efficacy data against DENV-2 and −4. J Virol. 2018;92(12). PubMed PMID: 29593041; PubMed Central PMCID: PMCPMC5974474. doi:10.1128/JVI.00440-18.
38. Palucka AK. Dengue virus and dendritic cells. Nat Med. 2000;6 (7):748–49. PubMed PMID: 10888920. doi:10.1038/77470.
39. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis. 2000;181(1):2–9. PubMed PMID: 10608744. doi:10.1086/315215.
40. Wijeratne DT, Fernando S, Gomes L, Jeewandara C, Ginneliya A, Samarasekara S, Wijewickrama A, Hardman CS, Ogg GS, Malavige GN, et al. Quantification of dengue virus specific T cell responses and correlation with viral load and clinical disease severity in acute dengue infection. PLoS Negl Trop Dis. 2018;12(10): e0006540. PubMed PMID: 30273352; PubMed Central PMCID: PMCPMC6181435. doi:10.1371/journal.pntd.0006540.
41. Singla M, Kar M, Sethi T, Kabra SK, Lodha R, Chandele A, Medigeshi GR, Williams M. Immune response to dengue virus infection in pediatric patients in New Delhi, India–Association of Viremia, inflammatory mediators and monocytes with disease severity. PLoS Negl Trop Dis. 2016;10(3):e0004497. PubMed PMID: 26982706; PubMed Central PMCID: PMCPMC4794248. doi:10.1371/journal.pntd.0004497.
42. Pal T, Dutta SK, Mandal S, Saha B, Tripathi A. Differential clinical symptoms among acute phase Indian patients revealed significant association with dengue viral load and serum IFN-gamma level. J Clin Virol. 2014;61(3):365–70. PubMed PMID: 25288310. doi:10.1016/j.jcv.2014.09.003.
43. Duong V, Ly S, Lorn Try P, Tuiskunen A, Ong S, Chroeung N, Lundkvist A, Leparc-Goffart I, Deubel V, Vong S, et al. Clinical and virological factors influencing the performance of a NS1 antigen-capture assay and potential use as a marker of dengue disease severity. PLoS Negl Trop Dis. 2011;5(7):e1244. PubMed PMID: 21811645; PubMed Central PMCID: PMCPMC3139664. doi:10.1371/journal.pntd.0001244.
44. Tang Y, Kou Z, Zhang F, Yao X, Liu S, Ma J, Zhou Y, Zhao W, Tang X, Jin X, et al. Both viremia and cytokine levels associate with the lack of severe disease in secondary dengue 1 infection among adult Chinese patients. PLoS One. 2010;5(12):e15631. PubMed PMID: 21206915; PubMed Central PMCID: PMCPMC3012067. doi:10.1371/journal.pone.0015631.
45. Wang WK, Chao DY, Kao CL, Wu HC, Liu YC, Li CM, Lin S-C, Ho S-T, Huang J-H, King -C-C. High levels of plasma dengue viral load during defervescence in patients with dengue hemorrhagic fever: implications for pathogenesis. Virology. 2003;305(2):330–38. PubMed PMID: 12573578. doi:10.1006/viro.2002.1704.
46. Fox A, Le NM, Simmons CP, Wolbers M, Wertheim HF, Pham TK, Ninh TTH, Lien TTM, Lien NT, Trung NV, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Ha Noi, Viet Nam. PLoS Negl Trop Dis. 2011;5 (3):e967. PubMed PMID: 21390156; PubMed Central PMCID: PMCPMC3046970. doi:10.1371/journal.pntd.0000967.
47. Endy TP. Human immune responses to dengue virus infection: lessons learned from prospective cohort studies. Front Immunol. 2014;5:183. PubMed PMID: 24795725; PubMed Central PMCID: PMCPMC4006038. doi:10.3389/fimmu.2014.00183.
48. Endy TP, Nisalak A, Chunsuttitwat S, Vaughn DW, Green S, Ennis FA, Rothman AL, Libraty DH. Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. J Infect Dis. 2004;189(6):990–1000. PubMed PMID: 14999601. doi:10.1086/382280.
49. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, Jiwariyavej V, Dulyachai W, Pengsaa K, Wartel TA, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet. 2012;380 (9853):1559–67. PubMed PMID: 22975340. doi:10.1016/S0140-6736(12)61428-7.
50. Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, Luong CQ, Rusmil K, Wirawan DN, Nallusamy R, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet. 2014;384(9951):1358–65. PubMed PMID: 25018116. doi:10.1016/S0140-6736(14)61060-6.
51. Villar L, Dayan GH, Arredondo-Garcia JL, Rivera DM, Cunha R, Deseda C, Reynales H, Costa MS, Morales-Ramírez JO, Carrasquilla G, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med. 2015;372(2):113–23. PubMed PMID: 25365753. doi:10.1056/NEJMoa1411037.
52. Guirakhoo F, Kitchener S, Morrison D, Forrat R, McCarthy K, Nichols R, Yoksan S, Duan X, Ermak TH, Kanesa-Thasan N, et al. Live attenuated chimeric yellow fever dengue type 2 (ChimeriVax-DEN2) vaccine: Phase I clinical trial for safety and immunogenicity: effect of yellow fever pre-immunity in induction of cross neutralizing antibody responses to all 4 dengue serotypes. Hum Vaccin. 2006;2(2):60–67. PubMed PMID: 17012873. doi:10.4161/hv.2.2.2555.
53. Morrison D, Legg TJ, Billings CW, Forrat R, Yoksan S, Lang J. A novel tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in flavivirus-naive adults. J Infect Dis. 2010;201 (3):370–77. PubMed PMID: 20059357. doi:10.1086/649916.
54. Capeding RZ, Luna IA, Bomasang E, Lupisan S, Lang J, Forrat R, Wartel A, Crevat D. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in the Philippines. Vaccine. 2011;29(22):3863–72. PubMed PMID: 21477675. doi:10.1016/j.vaccine.2011.03.057.
55. Poo J, Galan F, Forrat R, Zambrano B, Lang J, Dayan G. Live-attenuated tetravalent dengue vaccine in dengue-naive children, adolescents, and adults in Mexico City: randomized controlled phase 1 trial of safety and immunogenicity. Pediatr Infect Dis J. 2011;30(1):e9–17. PubMed PMID: 21042231. doi:10.1097/INF.0b013e3181fe05af.
56. Crevat D, Brion JD, Gailhardou S, Laot TM, Capeding MR. First experience of concomitant vaccination against dengue and MMR in Toddlers. Pediatr Infect Dis J. 2015;34(8):884–92. PubMed PMID: 25966916. doi:10.1097/INF.0000000000000752.
57. Qiao M, Shaw D, Forrat R, Wartel-Tram A, Lang J. Priming effect of dengue and yellow fever vaccination on the immunogenicity, infectivity, and safety of a tetravalent dengue vaccine in humans. Am J Trop Med Hyg. 2011;85(4):724–31. PubMed PMID: 21976579; PubMed Central PMCID: PMCPMC3183784. doi:10.4269/ajtmh.2011.10-0436.
58. Dayan GH, Galan-Herrera JF, Forrat R, Zambrano B, Bouckenooghe A, Harenberg A, Guy B, Lang J. Assessment of bivalent and tetravalent dengue vaccine formulations in

flavivirus-naive adults in Mexico. Hum Vaccin Immunother. 2014;10(10):2853–63. PubMed PMID: 25483647; PubMed Central PMCID: PMCPMC5443102. doi:10.4161/21645515.2014.972131.
59. Dayan GH, Thakur M, Boaz M, Johnson C. Safety and immunogenicity of three tetravalent dengue vaccine formulations in healthy adults in the USA. Vaccine. 2013;31(44):5047–54. PubMed PMID: 24021313. doi:10.1016/j.vaccine.2013.08.088.
60. Villar LA, Rivera-Medina DM, Arredondo-Garcia JL, Boaz M, Starr-Spires L, Thakur M, Zambrano B, Miranda MC, Rivas E, Dayan GH. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J. 2013;32(10):1102–09. PubMed PMID: 24067553. doi:10.1097/INF.0b013e31829b8022.
61. Tran NH, Luong CQ, Vu TQH, Forrat R, Lang J, Vu QD, Bouckenooghe A, Wartel TA. Safety and immunogenicity of recombinant, live attenuated tetravalent dengue vaccine (Dengvaxia®) in healthy Vietnamese adults and children. J Vaccines Vaccin. 2012;3(7):162.
62. Tran NH, Chansinghakul D, Chong CY, Low CY, Shek LP, Luong CQ, Fargo C, Wartel TA, Sun S, Skipetrova A, et al., Long-term immunogenicity and safety of tetravalent dengue vaccine (CYD-TDV) in healthy populations in Singapore and Vietnam: 4-year follow-up of randomized, controlled, phase II trials. Hum Vaccin Immunother. 2019;1–13. PubMed PMID: 30724660. doi:10.1080/21645515.2019.1578595.
63. Lanata CF, Andrade T, Gil AI, Terrones C, Valladolid O, Zambrano B, Saville M, Crevat D. Immunogenicity and safety of tetravalent dengue vaccine in 2-11 year-olds previously vaccinated against yellow fever: randomized, controlled, phase II study in Piura, Peru. Vaccine. 2012;30(41):5935–41. PubMed PMID: 22863660. doi:10.1016/j.vaccine.2012.07.043.
64. Leo YS, Wilder-Smith A, Archuleta S, Shek LP, Chong CY, Leong HN, Low CY, Oh M-LH, Bouckenooghe A, Wartel TA, et al. Immunogenicity and safety of recombinant tetravalent dengue vaccine (CYD-TDV) in individuals aged 2-45 y: Phase II randomized controlled trial in Singapore. Hum Vaccin Immunother. 2012;8(9):1259–71. PubMed PMID: 22894958; PubMed Central PMCID: PMCPMC3579907. doi:10.4161/hv.21224.
65. Harenberg A, Begue S, Mamessier A, Gimenez-Fourage S, Ching Seah C, Wei Liang A, Li Ng J, Yun Toh X, Archuleta S, Wilder-Smith A, et al. Persistence of Th1/Tc1 responses one year after tetravalent dengue vaccination in adults and adolescents in Singapore. Hum Vaccin Immunother. 2013;9(11):2317–25. PubMed PMID: 23839107; PubMed Central PMCID: PMCPMC3981840. doi:10.4161/hv.25562.
66. Dayan GH, Garbes P, Noriega F, Izoton de Sadovsky AD, Rodrigues PM, Giuberti C, Dietze R. Immunogenicity and safety of a recombinant tetravalent dengue vaccine in children and adolescents ages 9-16 years in Brazil. Am J Trop Med Hyg. 2013;89(6):1058–65. PubMed PMID: 24189367; PubMed Central PMCID: PMCPMC3854882. doi:10.4269/ajtmh.13-0304.
67. Dubey AP, Agarkhedkar S, Chhatwal J, Narayan A, Ganguly S, Wartel TA, Bouckenooghe A, Menezes J. Immunogenicity and safety of a tetravalent dengue vaccine in healthy adults in India: A randomized, observer-blind, placebo-controlled phase II trial. Hum Vaccin Immunother. 2016;12(2):512–18. PubMed PMID: 26291554; PubMed Central PMCID: PMCPMC5049724. doi:10.1080/21645515.2015.1076598.
68. Kirstein J, Douglas W, Thakur M, Boaz M, Papa T, Skipetrova A, Plennevaux E. Immunogenicity of the CYD tetravalent dengue vaccine using an accelerated schedule: randomised phase II study in US adults. BMC Infect Dis. 2018;18(1):475. 10.1186/s12879-018-3389-x. PubMed PMID: 30241510; PubMed Central PMCID: PMCPMC6150954. doi:10.1186/s12879-018-3109-6.
69. Coronel D, Garcia-Rivera EJ, Rivera M, Arredondo-Garcia JL, Dietze R, Perroud AP, Cortés M, Bonaparte M, Zhao J, Tila M, et al. Dengue vaccine booster in healthy adolescents and adults in Latin America: Evaluation 4-5 years after a primary 3-dose schedule. Pediatr Infect Dis J. 2019;38(5):e90–e5. PubMed PMID: 30986790. doi:10.1097/INF.0000000000002286.
70. Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T, Savarino S, Zambrano B, Moureau A, Khromava A, et al. Effect of dengue serostatus on dengue vaccine safety and efficacy. N Engl J Med. 2018;379:327–40. PubMed PMID: 29897841. doi:10.1056/NEJMoa1800820.
71. Arredondo-Garcia JL, Hadinegoro SR, Reynales H, Chua MN, Rivera Medina DM, Chotpitayasunondh T, Tran NH, Deseda CC, Wirawan DN, Cortés Supelano M, et al. Four-year safety follow-up of the tetravalent dengue vaccine efficacy randomized controlled trials in Asia and Latin America. Clin Microbiol Infect. 2018;24(7):755–63. PubMed PMID: 29408333. doi:10.1016/j.cmi.2018.01.018.
72. Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, Muhammad Ismail HIH, Reynales H, Limkittikul K, Rivera-Medina DM, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. 2015;373(13):1195–206. PubMed PMID: 26214039. doi:10.1056/NEJMoa1506223.
73. Torresi J, Heron LG, Qiao M, Marjason J, Chambonneau L, Bouckenooghe A, Boaz M, van der Vliet D, Wallace D, Hutagalung Y, et al. Lot-to-lot consistency of a tetravalent dengue vaccine in healthy adults in Australia: a randomised study. Vaccine. 2015;33(39):5127–34. PubMed PMID: 26279339. doi:10.1016/j.vaccine.2015.08.008.
74. Cortes M, Lopez P, Marquez V, Cortes C, Toro E, Noriega F, Zambrano B. Safety follow-up of a dengue vaccine when administered concomitantly with a yellow fever vaccine in healthy toddlers in Colombia. Pediatr Infect Dis J. 2018;37(11):1190–91. PubMed PMID: 30308599. doi:10.1097/INF.0000000000002172.
75. Lopez P, Lanata CF, Zambrano B, Cortes M, Andrade T, Amemiya I, Terrones C, Gil AI, Verastegui H, Marquez V, et al. Immunogenicity and safety of yellow fever vaccine (Stamaril) when administered concomitantly with a tetravalent dengue vaccine candidate in healthy toddlers at 12-13 Months of age in Colombia and Peru: a randomized trial. Pediatr Infect Dis J. 2016;35(10):1140–47. PubMed PMID: 27254034. doi:10.1097/INF.0000000000001250.
76. Hss AS, Koh MT, Tan KK, Chan LG, Zhou L, Bouckenooghe A, Crevat D, Hutagalung Y. Safety and immunogenicity of a tetravalent dengue vaccine in healthy children aged 2-11 years in Malaysia: a randomized, placebo-controlled, Phase III study. Vaccine. 2013;31(49):5814–21. PubMed PMID: 24135573. doi:10.1016/j.vaccine.2013.10.013.
77. Melo FIR, Morales JJR, De Los Santos AHM, Rivas E, Vigne C, Noriega F. Immunogenicity and safety of a booster injection of DTap-IPV//Hib (Pentaxim) administered concomitantly with tetravalent dengue vaccine in healthy toddlers 15-18 months of age in Mexico: a randomized trial. Pediatr Infect Dis J. 2017;36(6):602–08. PubMed PMID: 28067718. doi:10.1097/INF.0000000000001542.
78. Edelman R, Wasserman SS, Bodison SA, Putnak RJ, Eckels KH, Tang D, Kanesa-Thasan N, Vaughn DW, Innis BL, Sun W. Phase I trial of 16 formulations of a tetravalent live-attenuated dengue vaccine. Am J Trop Med Hyg. 2003;69(6 Suppl):48–60. PubMed PMID: 14740955. doi:10.4269/ajtmh.2003.69.48.
79. Sun W, Edelman R, Kanesa-Thasan N, Eckels KH, Putnak JR, King AD, Houng H-S, Tang D, Scherer JM, Hoke CH, et al. Vaccination of human volunteers with monovalent and tetravalent live-attenuated dengue vaccine candidates. Am J Trop Med Hyg. 2003;69(6 Suppl):24–31. PubMed PMID: 14740952. doi:10.4269/ajtmh.2003.69.6_suppl.0690024.
80. Sun W, Cunningham D, Wasserman SS, Perry J, Putnak JR, Eckels KH, Vaughn DW, Thomas SJ, Kanesa-Thasan N, Innis BL, et al. Phase 2 clinical trial of three formulations of tetravalent live-attenuated dengue vaccine in flavivirus-naive adults. Hum Vaccin. 2009;5(1):33–40. PubMed PMID: 18670195. doi:10.4161/hv.5.1.6348.
81. Guy B, Briand O, Lang J, Saville M, Jackson N. Development of the Sanofi Pasteur tetravalent dengue vaccine: one more step forward. Vaccine. 2015;33(50):7100–11. PubMed PMID: 26475445. doi:10.1016/j.vaccine.2015.09.108.

82. Jones JM, Lopez B, Adams L, Galvez FJ, Nunez AS, Santillan NA, Plante L, Hemme RR, Casal M, Hunsperger EA, et al. Binational dengue outbreak along the United States-Mexico Border - Yuma County, Arizona, and Sonora, Mexico, 2014. MMWR Morb Mortal Wkly Rep. 2016;65(19):495–99. PubMed PMID: 27196619. doi:10.15585/mmwr.mm6519a3.
83. Burattini MN, Lopez LF, Coutinho FA, Siqueira JB Jr., Homsani S, Sarti E, Massad E. Age and regional differences in clinical presentation and risk of hospitalization for dengue in Brazil, 2000-2014. Clinics (Sao Paulo). 2016;71(8):455–63. PubMed PMID: 27626476; PubMed Central PMCID: PMCPMC4975787. doi:10.6061/clinics/2016(08)08.
84. Hammond SN, Balmaseda A, Perez L, Tellez Y, Saborio SI, Mercado JC, Videa E, Rodriguez Y, Pérez MA, Cuadra R, et al. Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. Am J Trop Med Hyg. 2005;73(6):1063–70. PubMed PMID: 16354813.
85. Gamble J, Bethell D, Day NP, Loc PP, Phu NH, Gartside IB, Farrar JF, White NJ. Age-related changes in microvascular permeability: a significant factor in the susceptibility of children to shock?. Clin Sci (Lond). 2000;98(2):211–16. PubMed PMID: 10657278.
86. Bethell DB, Gamble J, Pham PL, Nguyen MD, Tran TH, Ha TH, Tran TN, Dong TH, Gartside IB, White NJ, et al. Noninvasive measurement of microvascular leakage in patients with dengue hemorrhagic fever. Clin Infect Dis. 2001;32(2):243–53. PubMed PMID: 11170914. doi:10.1086/318453.
87. Malavige GN, Ogg GS. Pathogenesis of vascular leak in dengue virus infection. Immunology. 2017;151(3):261–69. PubMed PMID: 28437586; PubMed Central PMCID: PMCPMC5461104. doi:10.1111/imm.12748.
88. Costa VV, Fagundes CT, Souza DG, Teixeira MM. Inflammatory and innate immune responses in dengue infection: protection versus disease induction. Am J Pathol. 2013;182(6):1950–61. PubMed PMID: 23567637. doi:10.1016/j.ajpath.2013.02.027.
89. Kurane I. Dengue hemorrhagic fever with special emphasis on immunopathogenesis. Comp Immunol Microbiol Infect Dis. 2007;30(5–6):329–40. PubMed PMID: 17645944. doi:10.1016/j.cimid.2007.05.010.
90. Halstead SB. Dengue. Lancet. 2007;370(9599):1644–52. PubMed PMID: 17993365. doi:10.1016/S0140-6736(07)61687-0.
91. Clapham H, Cummings DA, Nisalak A, Kalayanarooj S, Thaisomboonsuk B, Klungthong C, Fernandez S, Srikiatkhachorn A, Macareo LR, Lessler J, et al. Epidemiology of infant dengue cases illuminates serotype-specificity in the interaction between immunity and disease, and changes in transmission dynamics. PLoS Negl Trop Dis. 2015;9(12):e0004262. PubMed PMID: 26658730; PubMed Central PMCID: PMCPMC4684242. doi:10.1371/journal.pntd.0004262.
92. Nascimento EJM, George JK, Velasco M, Bonaparte MI, Zheng L, DiazGranados CA, Marques ETA, Huleatt JW. Development of an anti-dengue NS1 IgG ELISA to evaluate exposure to dengue virus. J Virol Methods. 2018;257:48–57. PubMed PMID: 29567514. doi:10.1016/j.jviromet.2018.03.007.
93. Olivera-Botello G, Coudeville L, Fanouillere K, Guy B, Chambonneau L, Noriega F, Jackson N. Tetravalent dengue vaccine reduces symptomatic and asymptomatic dengue virus infections in healthy children and adolescents aged 2-16 years in Asia and Latin America. J Infect Dis. 2016;214(7):994–1000. PubMed PMID: 27418050; PubMed Central PMCID: PMCPMC5021228. doi:10.1093/infdis/jiw297.
94. Kanesa-Thasan N, Edelman R, Tacket CO, Wasserman SS, Vaughn DW, Coster TS, Kim-Ahn GJ, Dubois DR, Putnak JR, King A, et al. Phase 1 studies of Walter Reed Army Institute of Research candidate attenuated dengue vaccines: selection of safe and immunogenic monovalent vaccines. Am J Trop Med Hyg. 2003;69(6 Suppl):17–23. PubMed PMID: 14740951. doi:10.4269/ajtmh.2003.69.17.
95. Huang CY, Butrapet S, Tsuchiya KR, Bhamarapravati N, Gubler DJ, Kinney RM. Dengue 2 PDK-53 virus as a chimeric carrier for tetravalent dengue vaccine development. J Virol. 2003;77(21):11436–47. PubMed PMID: 14557629; PubMed Central PMCID: PMCPMC229366. doi:10.1128/jvi.77.21.11436-11447.2003.
96. Osorio JE, Brewoo JN, Silengo SJ, Arguello J, Moldovan IR, Tary-Lehmann M, Powell TD, Livengood JA, Kinney RM, Huang CY-H, et al. Efficacy of a tetravalent chimeric dengue vaccine (DENVax) in Cynomolgus macaques. Am J Trop Med Hyg. 2011;84(6):978–87. PubMed PMID: 21633037; PubMed Central PMCID: PMCPMC3110349. doi:10.4269/ajtmh.2011.10-0592.
97. Bhamarapravati N, Sutee Y. Live attenuated tetravalent dengue vaccine. Vaccine. 2000;18 Suppl 2:44–47. PubMed PMID: 10821973. doi:10.1016/s0264-410x(00)00040-2.
98. Sabchareon A, Lang J, Chanthavanich P, Yoksan S, Forrat R, Attanath P, Sirivichayakul C, Pengsaa K, Pojjaroen-Anant C, Chokejindachai W, et al. Safety and immunogenicity of tetravalent live-attenuated dengue vaccines in Thai adult volunteers: role of serotype concentration, ratio, and multiple doses. Am J Trop Med Hyg. 2002;66(3):264–72. PubMed PMID: 12139219. doi:10.4269/ajtmh.2002.66.264.
99. Chanthavanich P, Luxemburger C, Sirivichayakul C, Lapphra K, Pengsaa K, Yoksan S, Sabchareon A, Lang J. Short report: immune response and occurrence of dengue infection in thai children three to eight years after vaccination with live attenuated tetravalent dengue vaccine. Am J Trop Med Hyg. 2006;75(1):26–28. PubMed PMID: 16837703. doi:10.4269/ajtmh.2006.75.1.0750026.
100. Trent D, Shin J, Hombach J, Knezevic I, Minor P; Group WHOW. WHO Working Group on technical specifications for manufacture and evaluation of dengue vaccines, Geneva, Switzerland, 11-12 May 2009. Vaccine. 2010;28(52):8246–55. PubMed PMID: 21036129. doi:10.1016/j.vaccine.2010.10.043
101. Roehrig JT, Hombach J, Barrett AD. Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. Viral Immunol. 2008;21(2):123–32. 10.1089/vim.2008.0007. PubMed PMID: 18476771.
102. Thomas SJ. Developing a dengue vaccine: progress and future challenges. Ann N Y Acad Sci. 2014;1323:140–59. PubMed PMID: 24689974. doi:10.1111/nyas.12413.
103. Thomas SJ, Endy TP. Critical issues in dengue vaccine development. Curr Opin Infect Dis. 2011;24(5):442–50. PubMed PMID: 21799408. doi:10.1097/QCO.0b013e32834a1b0b.
104. Thomas SJ, Rothman AL. Trials and tribulations on the path to developing a dengue vaccine. Am J Prev Med. 2015;49(6 Suppl 4):S334–44. PubMed PMID: 26590433. doi:10.1016/j.amepre.2015.09.006.
105. Torresi J, Richmond PC, Heron LG, Qiao M, Marjason J, Starr-Spires L, van der Vliet D, Jin J, Wartel TA, Bouckenooghe A. Replication and excretion of the live attenuated tetravalent dengue Vaccine CYD-TDV in a flavivirus-naive adult population: assessment of vaccine viremia and virus shedding. J Infect Dis. 2017;216(7):834–41. PubMed PMID: 28968794. doi:10.1093/infdis/jix314.
106. Dorigatti I, Aguas R, Donnelly CA, Guy B, Coudeville L, Jackson N, Saville M, Ferguson NM. Modelling the immunological response to a tetravalent dengue vaccine from multiple phase-2 trials in Latin America and South East Asia. Vaccine. 2015;33(31):3746–51. PubMed PMID: 26051515; PubMed Central PMCID: PMCPMC4504002. doi:10.1016/j.vaccine.2015.05.059.
107. Henein S, Swanstrom J, Byers AM, Moser JM, Shaik SF, Bonaparte M, Jackson N, Guy B, Baric R, de Silva AM. Dissecting antibodies induced by a Chimeric Yellow Fever-Dengue, Live-Attenuated, Tetravalent Dengue Vaccine (CYD-TDV) in naive and dengue-exposed individuals. J Infect Dis. 2017;215(3):351–58. PubMed PMID: 27932620. doi:10.1093/infdis/jiw576.
108. Moodie Z, Juraska M, Huang Y, Zhuang Y, Fong Y, Carpp LN, Self SG, Chambonneau L, Small R, Jackson N, et al. Neutralizing antibody correlates analysis of tetravalent dengue vaccine efficacy trials in Asia and Latin America. J Infect Dis. 2018;217(5):742–53. PubMed PMID: 29194547; PubMed Central PMCID: PMCPMC5854020. doi:10.1093/infdis/jix609.
109. Lecouturier V, Berry C, Saulnier A, Naville S, Manin C, Girerd-Chambaz Y, Crowe JE, Jackson N, Guy B. Characterization of

recombinant yellow fever-dengue vaccine viruses with human monoclonal antibodies targeting key conformational epitopes. Vaccine. 2018. PubMed PMID: 29706291. doi:10.1016/j.vaccine.2018.04.065

110. Friberg H, Beaumier CM, Park S, Pazoles P, Endy TP, Mathew A, Currier JR, Jarman RG, Anderson KB, Hatch S, et al. Protective versus pathologic pre-exposure cytokine profiles in dengue virus infection. PLoS Negl Trop Dis. 2018;12(12):e0006975. PubMed PMID: 30557313; PubMed Central PMCID: PMCPMC6312351. doi:10.1371/journal.pntd.0006975.
111. Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. Semin Immunopathol. 2017;39(5):563–74. 10.1007/s00281-017-0625-1. PubMed PMID: 28401256; PubMed Central PMCID: PMCPMC5496927. doi:10.1007/s00281-017-0625-1.
112. Rothman AL, Medin CL, Friberg H, Currier JR. Immunopathogenesis versus protection in dengue virus infections. Curr Trop Med Rep. 2014;1(1):13–20. PubMed PMID: 24883262; PubMed Central PMCID: PMCPMC4036645. doi:10.1007/s40475-013-0009-0.
113. Kurane I, Matsutani T, Suzuki R, Takasaki T, Kalayanarooj S, Green S, Rothman AL, Ennis FA. T-cell responses to dengue virus in humans. Trop Med Health. 2011;39(4 Suppl):45–51. PubMed PMID: 22500136; PubMed Central PMCID: PMCPMC3317604. doi:10.2149/tmh.2011-S09.
114. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol. 2011;11(8):532–43. PubMed PMID: 21760609. doi:10.1038/nri3014.
115. Friberg H, Burns L, Woda M, Kalayanarooj S, Endy TP, Stephens HAF, Green S, Rothman AL, Mathew A. Memory CD8 + T cells from naturally acquired primary dengue virus infection are highly cross-reactive. Immunol Cell Biol. 2011;89(1):122–29. PubMed PMID: 20421879; PubMed Central PMCID: PMCPMC2929403. doi:10.1038/icb.2010.61.
116. Rothman AL. Cellular immunology of sequential dengue virus infection and its role in disease pathogenesis. Curr Top Microbiol Immunol. 2010;338:83–98. PubMed PMID: 19802580. doi:10.1007/978-3-642-02215-9_7.
117. Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, Chambonneau L, Morrisson DN, Shaw D, Qiao M, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine. 2008;26(45):5712–21. PubMed PMID: 18762226. doi:10.1016/j.vaccine.2008.08.019.
118. Guy B, Jackson N. Dengue vaccine: hypotheses to understand CYD-TDV-induced protection. Nat Rev Microbiol. 2016;14(1):45–54. PubMed PMID: 26639777. doi:10.1038/nrmicro.2015.2.
119. Anderson KB, Endy TP, Thomas SJ. The dynamic role of dengue cross-reactive immunity: changing the approach to defining vaccine safety and efficacy. Lancet Infect Dis. 2018;18(10):e333–e8. PubMed PMID: 29784457. doi:10.1016/S1473-3099(18)30126-9.
120. Oktarianti R, Senjarini K, Hayano T, Fatchiyah F. Proteomic analysis of immunogenic proteins from salivary glands of Aedes aegypti. J Infect Public Health. 2015;8(6):575–82. PubMed PMID: 26054892. doi:10.1016/j.jiph.2015.04.022.
121. Doucoure S, Mouchet F, Cornelie S, Drame PM, D'Ortenzio E, DeHecq JS, Remoue F. Human antibody response to Aedes albopictus salivary proteins: a potential biomarker to evaluate the efficacy of vector control in an area of Chikungunya and Dengue virus transmission. Biomed Res Int. 2014;2014:746509. PubMed PMID: 24822216; PubMed Central PMCID: PMCPMC4005104. doi:10.1155/2014/746509.
122. Machain-Williams C, Mammen MP Jr., Zeidner NS, Beaty BJ, Prenni JE, Nisalak A, Blair CD. Association of human immune response to Aedes aegypti salivary proteins with dengue disease severity. Parasite Immunol. 2012;34(1):15–22. PubMed PMID: 21995849; PubMed Central PMCID: PMCPMC3240707. doi:10.1111/j.1365-3024.2011.01339.x.
123. Velumani S, Toh YX, Balasingam S, Archuleta S, Leo YS, Gan VC, Thein TL, Wilder-Smith A, Fink K. Low antibody titers 5 years after vaccination with the CYD-TDV dengue vaccine in both pre-immune and naive vaccinees. Hum Vaccin Immunother. 2016;12(5):1265–73. PubMed PMID: 26889737; PubMed Central PMCID: PMCPMC4963049. doi:10.1080/21645515.2015.1126012.
124. Vigne C, Dupuy M, Richetin A, Guy B, Jackson N, Bonaparte M, Hu B, Saville M, Chansinghakul D, Noriega F, et al. Integrated immunogenicity analysis of a tetravalent dengue vaccine up to 4 y after vaccination. Hum Vaccin Immunother. 2017;1–13. PubMed PMID: 28598256. doi:10.1080/21645515.2017.1333211
125. Rabaa MA, Girerd-Chambaz Y, Duong Thi Hue K, Vu Tuan T, Wills B, Bonaparte M, van der Vliet D, Langevin E, Cortes M, Zambrano B, et al. Genetic epidemiology of dengue viruses in phase III trials of the CYD tetravalent dengue vaccine and implications for efficacy. Elife. 2017;6. PubMed PMID: 28871961; PubMed Central PMCID: PMCPMC5584992. doi:10.7554/eLife.24196.
126. Juraska M, Magaret CA, Shao J, Carpp LN, Fiore-Gartland AJ, Benkeser D, Girerd-Chambaz Y, Langevin E, Frago C, Guy B, et al. Viral genetic diversity and protective efficacy of a tetravalent dengue vaccine in two phase 3 trials. Proc Natl Acad Sci U S A. 2018;115(36):E8378–E87. PubMed PMID: 30127007; PubMed Central PMCID: PMCPMC6130398. doi:10.1073/pnas.1714250115.