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Development of the Sanofi Pasteur tetravalent dengue vaccine: One more step forward

Bruno Guy[a,*], Olivier Briand[b], Jean Lang[a], Melanie Saville[a], Nicholas Jackson[a]

[a] Research and Development, Sanofi Pasteur, 69007 Lyon, France
[b] Industrial Operations, Sanofi Pasteur, 69007 Lyon, France

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ABSTRACT

Sanofi Pasteur has developed a recombinant, live-attenuated, tetravalent dengue vaccine (CYD-TDV) that is in late-stage development. The present review summarizes the different steps in the development of this dengue vaccine, with a particular focus on the clinical data from three efficacy trials, which includes one proof-of-concept phase IIb (NCT00842530) and two pivotal phase III efficacy trials (NCT01373281 and NCT01374516). Earlier studies showed that the CYD-TDV candidate had a satisfactory safety profile and was immunogenic across the four vaccine serotypes in both *in vitro* and *in vivo* preclinical tests, as well as in initial phase I to phase II clinical trials in both flavivirus-naïve and seropositive individuals. Data from the 25 months (after the first injection) active phase of the two pivotal phase III efficacy studies shows that CYD-TDV (administered at 0, 6, and 12 months) is efficacious against virologically-confirmed disease (primary endpoint) and has a good safety profile. Secondary analyses also showed efficacy against all four dengue serotypes and protection against severe disease and hospitalization. The end of the active phases in these studies completes more than a decade of development of CYD-TDV, but considerable activities and efforts remain to address outstanding scientific, clinical, and immunological questions, while preparing for the introduction and use of CYD-TDV. Additional safety observations were recently reported from the first complete year of hospital phase longer term surveillance for two phase 3 studies and the first and second completed years for one phase 2b study, demonstrating the optimal age for intervention from 9 years. Dengue is a complex disease, and both short-term and long-term safety and efficacy will continue to be addressed by ongoing long-term follow-up and future post-licensure studies.
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1. Introduction

The month of November 2014 saw the successful achievement of a critical phase of an ambitious and challenging program that had been launched 20 years before, supported by an overall investment of more than one billion Euros, and which mobilized more than 1000 people in a large number of countries across different continents: for the first time in human history, a robot successfully landed on a comet. Despite difficulties and uncertainties, Philae and Rosetta so far have achieved most of their initial goals and have already brought a significant contribution to space exploration.

The development of the Sanofi Pasteur's recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine (TDV), often referred to as the CYD dengue vaccine or CYD-TDV, presents many similarities with the long-standing effort represented by the European Rosetta project, having started at the same time in the mid-90s, required a similar investment, and mobilized similar numbers of collaborators from multigeopgraphic and multidisciplinary teams, within and outside the company, and for which success could only be established at the end of development. While it still represents a large effort necessitating the acquisition of data in pivotal efficacy trials, the present review aims to summarize the different steps of this vaccine's development, focusing on the clinical data acquired in the last four years from three efficacy trials—one proof-of-concept phase IIb and two pivotal phase III efficacy trials. It will thus update the previous reviews published in *Vaccine* in 2010 and 2011 [1,2].

The initial steps of the preclinical and clinical development of CYD-TDV will be briefly covered, followed by a more detailed description and comparison of the three efficacy trials, where results of the active phase were obtained in 2012 and in 2014 [3–6].

Abbreviations: CYD-TDV or TDV, recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine; DENV-1–4, dengue virus serotypes 1–4; $PRNT_{50}$, 50% reduction dengue viral plaques as determined with the plaque-reduction neutralization test; WHO, World Health Organization.

* Corresponding author at: Research and Development, Sanofi Pasteur, 2 av. Pont Pasteur, 69007 Lyon, France. Tel.: +33 04 37 66 98 17.
E-mail address: bruno.guy@sanofipasteur.com (B. Guy).

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Beyond the observed results, these ongoing trials (now in the hospital surveillance period) have generated several questions and lines of investigations, which will be addressed. Finally, while a critical phase has now been completed, it is important to consider what remains to ensure implementation of the vaccine that will bring the highest benefit to human health, which will also be described.

2. Early development

Previous reviews have described in detail the results of initial investigations that aimed to characterize the CYD-TDV at both preclinical and early clinical development stages, in order to evaluate potential risks in line with a Development Risk Management Plan at the onset of CYD-TDV availability [1,2]. These investigations addressed the following points and questions, with conclusions presented in Fig. 1:

- Genetic and phenotypic stability [7–9].
- Post-translational modifications such as glycosylation [10].
- Preclinical evaluation of immunogenicity: *in vitro* [11–13] and *in vivo* [14–16].
- Non-clinical safety [17].
- Theoretical risks: transmission by arthropod vectors [18,19], reversion to virulence [20], recombination with a wild type (flavi)virus [21–23], viscerotropism [11,24], sensitization/antibody-dependent enhancement (ADE) [25].
- Initial clinical evaluation: reactogenicity/safety and immunogenicity, addressing both neutralizing antibody [26–33] and T-cell responses [34–36].

These investigations demonstrated that the CYD-TDV candidate had satisfactory safety and immunogenicity in both *in vitro* and *in vivo* preclinical tests, as well as in initial phase I and phase II clinical trials in both flavivirus naïve and seropositive individuals. Furthermore, both humoral and cellular responses were induced in humans against all four dengue virus serotypes (DENV-1–4) of the vaccine.

These potential risks hypothesized as being associated with these chimeric vaccine viruses have been explored in depth. They have been identified based on current knowledge in the dengue field, and also in agreement with the European Medicines Agency (EMA) guidelines on recombinant vaccines [37]. A recently proposed standardized template for a risk-benefit assessment of vaccines based on a yellow fever backbone is a key consideration in the comparison of such vaccine technologies [38]. For each of the potential risks, a risk-minimization action plan was defined and data generated, which were subsequently reviewed with the World Health Organization (WHO), Pan American Health Organization, Centers for Disease Control and Prevention, key opinion leaders, and regulatory agencies.

3. Industrial development

Scale up and industrialization were initiated very early on in the development program—in parallel with the preclinical phase and clinical phase I—to ensure that the demand for the vaccine to be used in clinical phase III could be met, as well as the future demand for the licensed vaccine. The production process has been summarized previously [2], and was set up to ensure a reliable and consistent supply of virus and cells at the industrial level.

As early as 2006, the production process was transferred to Industrial Operations and a production facility was dedicated at the Marcy L'Etoile site that was equipped with industrial-scale biogenerators to produce Vero cells and virus. Briefly, the four vaccine viruses are produced from four virus seed lots using an identical manufacturing process for each serotype. Banking systems for serum-free Vero cells were established to produce master and working viral seeds and cells, allowing reliable and consistent supply of virus and cells, respectively. The vaccines and cells are characterized and tested for safety in accordance with WHO, European and US guidelines [39]. All the tests undertaken are part of a control strategy designed to ensure product quality and consistency. These included quality control specification, product characterization, adherence to good manufacturing practices, validated manufacturing process, raw-materials testing, in-process testing, and stability testing. The quality control specification, which is typical for a live, attenuated, viral vaccine, based on current regulations and guidelines, mainly determined the purity, safety, and potency of the vaccine [39]. Due to the use of Sanofi Pasteur's serum-free Vero cell banks for both cell and viral culture, the CYD-TDV manufacturing process includes no raw materials of animal origin; neither does the vaccine contain any preservatives, adjuvants, or antibiotics. A proprietary stabilizer is present in the finished product, which has been shown to have excellent stability: accelerated stability studies have shown that the vaccine from the phase III lots of CYD-TDV (unidose presentation) was stable up to 1 month at $25 \pm 2\,^{\circ}C$, and that the viral titer decreased by less than $0.5\ \log_{10}\ CCID_{50}$ (the 50% cell culture infective dose) after 7 days at $+37 \pm 2$ C. Reconstituted vaccine was found to be stable for up to 6 h at $+5 \pm 3\,^{\circ}C$.

In 2008, 4 years before the first results of the phase IIb clinical trials, it was decided to establish a new vaccine production site at Neuville sur Saone, France, in anticipation of future vaccine needs and to be able to minimize the vaccine-to-vaccination gap after regulatory approval. This represented a €300 million investment, consistent with the continuous efforts made by the Sanofi Pasteur teams over the past 20 years toward the development of a safe and efficacious dengue vaccine. Three new dedicated facilities (utilities, quality control, and production) have been built on this site using quality by design principles, and fitted with state of the art technology equipment. The Neuville sur Saone production facilities scales up the Marcy l'Etoile facility and can produce up to 100 million doses per year of vaccine virus (drug substance, packaged in monodose or multidose vials). The new site has gone through the qualification and validation steps, and consistent lots have already been produced at this site that will ensure the availability of the vaccine on an industrial scale over the coming years.

Another important Sanofi Pasteur site, in Val de Reuil, France, is also involved in the production of CYD-TDV. Here, the vaccine is formulated, filled, lyophilized, and packaged before release. The final product can then be shipped from Val de Reuil to all over the world (through distribution facilities).

4. Recent clinical development: phase IIb (CYD23) and phase III (CYD14 and CYD15) efficacy trials

The clinical development of CYD-TDV has complied with the International Conference on Harmonization guidance for industry, as well as the US Food and Drug Administration and European Medicines Agency guidelines for new vaccines, and the WHO Technical Report Series 932 guidelines for the production and quality control of live candidate tetravalent dengue virus vaccines [39]. This required consideration of specific development challenges (Fig. 1), in addition to the lack of a predictive immunocompetent animal disease model. These challenges include but are not limited to: (i) the need to induce an adequate immune response to all four serotypes; (ii) the current absence of a correlate and threshold of protection, and thus the need to demonstrate clinical efficacy; (iii) the need to demonstrate long-term safety; (iv) the potential risks after vaccination of sensitization to severe dengue infection and of

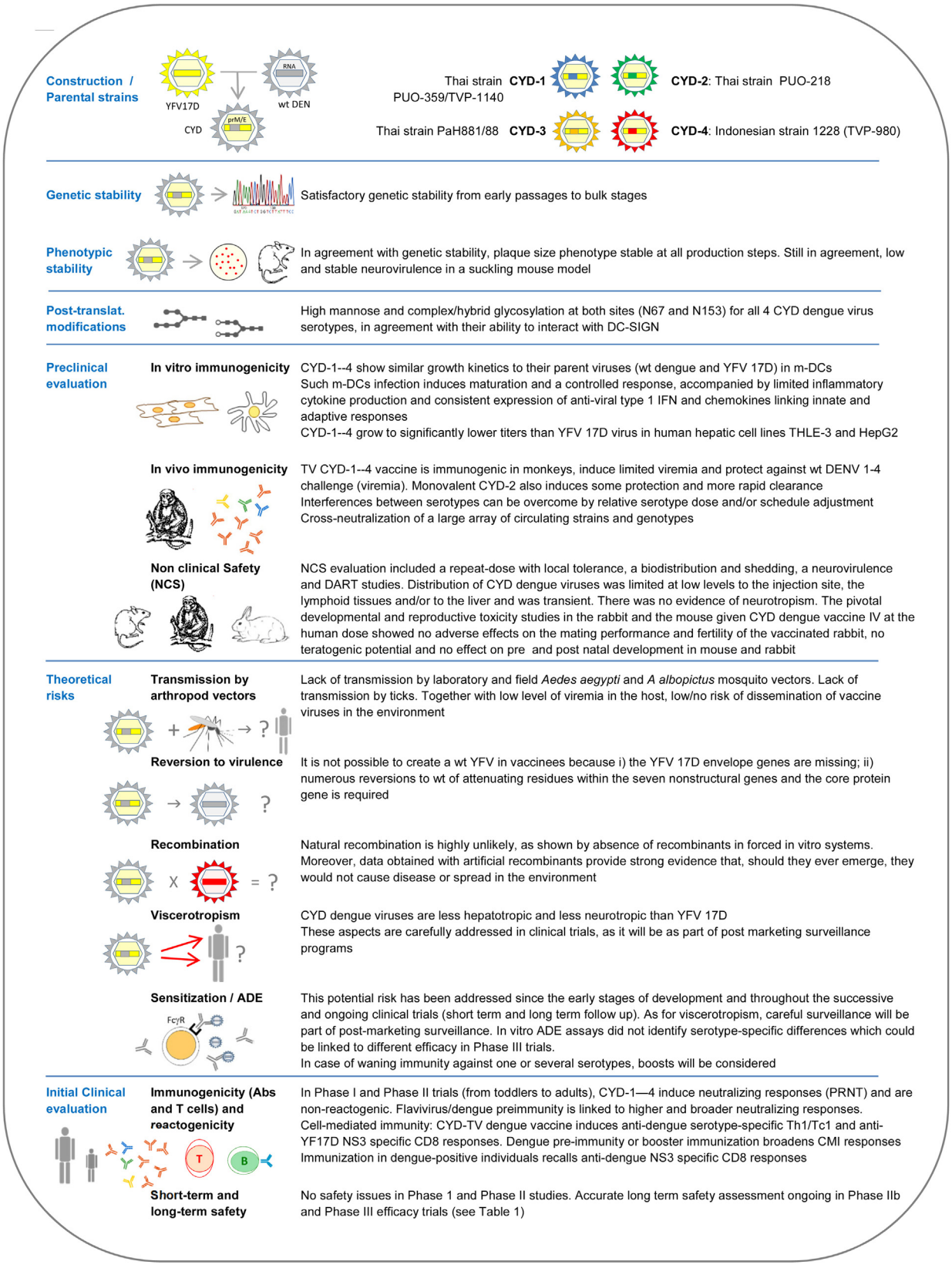

Fig. 1. The preclinical and early clinical development of the CYD-TDV. Investigations addressed the genetic and phenotypic stability of the CYD dengue viruses, their post-translational modifications, and their *in vitro* and *in vivo* immunogenicity in primary/transformed cells and in monkeys. Non-clinical safety (NCS) was assessed in mice, monkeys, and rabbits. Theoretical risks were assessed in various *in vitro* and *in vivo* models, and the B and T cell responses in phase I and phase II trials. Abbreviations: ADE, antibody-dependent enhancement; CMI, cell-mediated immunity; CYD, chimeric yellow fever dengue; CYD-1–4, dengue vaccine serotypes 1–4; DART, developmental and reproductive toxicology; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, also known as CD209; DENV 1–4, dengue virus serotypes 1–4; IFN, interferon; IV, intravenous; m-DCs, monocyte-derived dendritic cells; NS3, non-structural antigens; PRNT, plaque-reduction neutralization test; wt, wild type; YFD 17D, yellow fever virus strain 17D.

acute viscerotropic disease and neurotropic disease, which are very rare serious adverse events after vaccination with the yellow fever virus (YFV)-17D vaccine; and (v) the need to comply with genetically modified organisms regulations. Additional complexity for clinical development included differential background flavivirus seroprevalence rates between regions, necessitating that trials be conducted in parallel at numerous sites across different countries and in different continents. In this regard, an additional challenge was to ensure that there was appropriate clinical trial infrastructure in place across these various sites.

As mentioned previously, the CYD-TDV candidate had a satisfactory safety profile and good immunogenicity in initial phase I and phase II trials, and in subsequent phase III trials [4,5,32]. These trials were conducted over ten years on several continents in children, adolescents and adults with a diverse flavivirus infection and vaccination history, as a prelude to the efficacy trials. Overall, nearly 30,000 participants have received at least one dose of the CYD-TDV, and in compliance with the WHO guidelines, at each phase of clinical development many participants have been and are still being followed up on the long-term (3–5 years), in order to monitor long-term safety including for potential severe dengue.

As part of the clinical development of the vaccine, three efficacy trials were undertaken, the results from which have been published in the past two years. These included a proof-of-concept phase IIb efficacy trial in Thailand [3], and two pivotal phase III efficacy trials in Asia [4] and Latin America [5]. Indeed, the first priority was to develop the vaccine in endemic countries of Asia-Pacific, Latin America, and the Caribbean to address the unmet medical need in these regions. The main results of these three trials are presented in Table 1.

4.1. First proof-of-concept efficacy study in Thailand: CYD23 phase IIb (NCT00842530)

The proof-of-concept, phase IIb clinical efficacy study was an observer-masked, randomized, controlled, single-center trial in healthy Thai schoolchildren aged 4–11 years, who were randomly assigned (2:1) to receive three injections of CYD-TDV or control (rabies vaccine or placebo) at 0, 6, and 12 months in one high incidence area in Ratchaburi, Thailand [3]. Participants were actively followed up until 25 months (active phase). All acute febrile illnesses were tested for dengue, and viremia was confirmed by serotype-specific reverse transcriptase polymerase chain reaction (RT-PCR) and non-structural protein 1 enzyme-linked immunosorbent assay. The primary objective was to assess the protective efficacy against virologically-confirmed, symptomatic dengue, irrespective of severity or serotype occurring at least 1 month after the third injection and up to the end of the active phase (per-protocol analysis). A total of 4002 participants were assigned to vaccine ($n = 2669$) or control ($n = 1333$), with 3673 participants overall included in the primary analysis (vaccine, $n = 2452$; control, $n = 1221$). During the study, 134 cases of virologically confirmed dengue occurred. The incidence of dengue disease was high in the trial (dominated by serotype 2), and the more than expected number of cases enabled endpoints that would have otherwise not been possible to consider, including serotype-specific efficacy, to be assessed. A similar situation (involving all 4 serotypes) was encountered in the two subsequent phase III trials (CYD14 and CYD15 studies; see below).

In the CYD23 study, vaccine efficacy according to the primary endpoint was 30.2% (95% confidence interval [CI]: −13.4 to 56.6), but differed by serotype. In the intent-to-treat population (*i.e.* all participants who received ≥1 injection, analysis performed from the first dose, month 0–25), the efficacy observed for DENV-1 was 61.2% (95% CI: 17.4–82.1), 81.9% (95% CI: 38.8–95.8) for DENV-3, and 90.0% (95% CI: 10.6–99.8) for DENV-4. However, for the serotype 2, the predominant serotype, efficacy was only 3.5% (95% CI: −59.8 to 40.5). The lack of observed efficacy against DENV-2 occurred despite satisfactory immunogenicity as determined by the plaque-reduction neutralization test ($PRNT_{50}$) levels 28 days after the third dose, which were at least similar to the other serotypes (Table 1).

The dengue vaccine was well tolerated, with no safety signals after 2 years of active follow-up after the first dose. The subsequent hospital-phase surveillance for the phase IIb study (the CYD57 study) continues until 2016. For the first time, these data showed that a vaccine against dengue was feasible, but it raised several important questions; in particular, the link between $PRNT_{50}$ levels and protection, and the importance of varying serotype-specific efficacy. Both topics will be addressed later in this review. One has also to consider some differences between the phase IIb and Phase III efficacy trials (Table 1): the phase IIb was a single center study, the definition of acute febrile illness was slightly different, and serotype 2 was the dominant circulating serotype in the study.

4.2. Pivotal phase III efficacy studies in Asia and Latin America: CYD14 (NCT01373281) and CYD15 (NCT01374516)

The active phases of two, pivotal, phase III efficacy studies were undertaken in children aged 2–14 years in Asia [4], and in children and adolescents aged 9–16 years in Central and South America, using consistency lots at final industrial scale [5]. Each trial included five endemic countries (involving 11 sites in Asia and 22 sites in Latin America) and, as in CYD23, examined the efficacy of a three-dose schedule (0, 6, and 12 months) of CYD-TDV to reduce symptomatic, virologically-confirmed dengue during a period of 12 months starting 28 days after the third dose (Fig. 2). In both trials, the primary endpoint was for the lower bound of the 95% confidence interval (CI) of vaccine efficacy to be greater than 25% (*versus* 0% in the previous Phase IIb). The active phase of these two trials has now been completed, providing efficacy and safety results from the first 25 months following the initial immunization (Table 1).

In the Asian trial (CYD14), efficacy against virologically-confirmed dengue was 56.5% (95% CI: 43.8–66.4) in the per protocol group, irrespective of disease severity against any serotype. Similarly, in the Central and South American trial (CYD15), overall efficacy against virologically-confirmed dengue was 60.8% (95% CI: 52.0–68.0). Thus, both trials successfully met their primary endpoint (*i.e.* lower limit of the 95% CI for efficacy was >25%) with comparable overall efficacy between the two studies. Secondary descriptive analyses in the Asian study showed that all four dengue serotypes contributed to the overall efficacy during the active phase, although the efficacy against serotype 2 was inconclusive, as the lower limit of the 95% CI for vaccine efficacy included zero in the per protocol analysis. In the Central and South American trial, efficacy was highest against serotype 4 and lowest for serotype 2, but was conclusive against all four serotypes (*i.e.* lower limit of the 95% CI of vaccine efficacy was above zero in both the per-protocol and the intent-to-treat analyses). To provide a broader perspective, pooled efficacy analyses for both these trials demonstrated conclusive efficacy against all four serotypes [40]. The varying levels of efficacy against the four serotypes further confirm the importance of having sufficiently large field efficacy trials to capture an adequate number of cases with which to address such secondary endpoints, or to demonstrate protection against hospitalization and severe disease (see below).

During the active phase, both trials showed higher efficacy against severe disease and hospitalization for dengue, as well as trends toward reduced symptomatology from dengue after vaccination (in CYD14, 56.5% overall efficacy against dengue disease *vs* 67.2% against hospitalization and 80.0% against dengue hemorrhagic fever; in CYD15, 60.8% overall efficacy against dengue

Table 1
Design and main results of the active phase of Phase IIb and pivotal Phase III efficacy studies with CYD-TDV.

	CYD23 (NCT00842530) [3]	CYD14 (NCT01373281) [4]	CYD15 (NCT01374516) [5]
Study design			
Primary objective	To assess the efficacy of CYD dengue vaccine after 3 vaccine doses at 0, 6, 12 months in preventing symptomatic virologically-confirmed dengue cases (>28 days PD3), regardless of severity, due to any of the 4 serotypes		
Age at inclusion (years)	4–11	2–14	9–16
Acute febrile illness	Fever for at least 1 day (temperature ≥37.5 °C measured at least twice at least 4 h apart)	Temperature ≥38 °C on at least 2 consecutive days	
Virological confirmation	Dengue RT-PCR and/or dengue NS1 ELISA Ag test	Dengue RT-PCR and/or dengue NS1 ELISA Ag test Simplexa dengue RT-PCR used for ST identification	
Per protocol set	Per protocol set for efficacy (PPSE) included all participants who received 3 injections and who were compliant with the protocol, and was used to assess the primary objective		
Power	80%	90%	90%
Lower limit 95% CI VE	>0%	>25%	>25%
Assumed incidence	1.3%	1.3%	0.64%
Sample size (vaccinees/Placebos: 2/1)	4002	10,275	20,869
Countries	Thailand (1 site)	Thailand, Philippines, Vietnam, Indonesia, Malaysia (11 sites)	Brazil, Mexico, Honduras, Columbia, Puerto Rico (22 sites)
Epidemiology			
Observed attack rate	2.6%	4.7%	2.9%
Cases	134	595	662
Serotype distribution	ST1: 29%; ST2: 43%; ST3: 18%; ST4: 8%; Unserotyped: 1%	ST1: 39%; ST2: 23%; ST3: 13%; ST4: 22%; Unserotyped: 3%	ST1: 28%; ST2: 21%; ST3: 27%; ST4: 21%; Unserotyped: 3%
Per protocol VE (95% CI)[a]			
Any serotype	**30.2%** (−13.4, 56.6)	**56.5%** (43.8, 66.4)	**60.8%** (52.0, 68.0)
Serotype-specific	ST1: **55.3%** (−22, 83.9) ST2: **15.6%** (−58, 53.6) ST3: **75.1%** (−375, 99.6) ST4: **100%** (24.3, 100)	ST1: **50.0%** (24.6, 66.8) ST2: **35.0%** (−9.2, 61) ST3: **78.4%** (52.9, 90.8) ST4: **75.3%** (54.5, 87)	ST1: **50.3%** (29.1, 65.2) ST2: **42.3%** (14, 61.1) ST3: **74.0%** (61.9, 82.4) ST4: **77.7%** (60.2, 88)
ITT VE (95% CI)[b]			
Any serotype	**34.9%** (6.7, 54.3)	**54.8%** (46.8, 61.7)	**64.7%** (58.7, 69.8)
Serotype-specific	ST1: **61.2%** (17.4, 82.1) ST2: **3.5%** (−58, 40.5) ST3: **81.9%** (38.8, 95.8) ST4: **90%** (10.6, 99.8)	ST1: **54.5%** (40.9, 64.9) ST2: **34.70%** (10.4, 52.3) ST3: **65.2%** (43.3, 78.9) ST4: **72.4%** (58.8, 81.7)	ST1: **54.8%** (40.2, 65.9) ST2: **50.2%** (31.8, 63.6) ST3: **74.2%** (63.9, 81.7) ST4: **80.9%** (70.9, 87.7)
VE (95% CI)			
Severe disease[c]	**49.9%** (−590.6, 96.4)	**70.0%** (35.7, 86.6	**95.5%** (68.8, 99.9)
DHF[d]	**49.9%** (−590.6, 96.4)	**80.0%** (52.7, 92.4)	**95.0%** (64.9, 99.9)
Hospitalization	**46.8%** (9.3, 68.7)	**67.2%** (50.3, 78.6)	**80.3%** (64.7, 89.5)
Immunogenicity/PRNT GMTs (95% CI)			
Baseline	ST1: **42.8** (30.7, 59.6) ST2: **56.8** (40.3, 80.1) ST3: **31.5** (24.2, 41.0) ST4: **28.1** (21.7, 36.4)	ST1: **38.3** (33.8, 43.5) ST2: **55.3** (48.7, 62.9) ST3: **40.1** (35.6, 45.1) ST4: **25.3** (22.9, 28.0)	ST1: **128** (112, 145) ST2: **138** (123, 156) ST3: **121** (108, 136) ST4: **43.6** (39.6, 48.0)
28d Post Dose 3	ST1: **155** (116, 207) ST2: **358** (283, 453) ST3: **351** (289, 428) ST4: **151** (128, 178)	ST1: **166** (150, 183) ST2: **355** (327, 386) ST3: **207** (189, 226) ST4: **151** (141, 162)	ST1: **395** (353, 441) ST2: **574** (528, 624) ST3: **508** (465, 555) ST4: **241** (226; 258)

Abbreviations: Ag, antigen; CI, confidence interval; DHF, dengue hemorrhagic fever; ELISA, enzyme-linked immunosorbant assay; GMTs, geometric mean titers; NS1, non-structural antigen 1; PRNT, plaque-reduction neutralization test; RT-PCR, reverse transcriptase polymerase chain reaction; ST, serotype; VE, vaccine efficacy. Bold numbers are point estimates.

[a] Per protocol (PP) analysis: assessment of protective efficacy against virologically confirmed, symptomatic dengue, irrespective of severity or serotype, occurring 1 month after the third injection and through to the end of the 25-month active phase (see Fig. 2).

[b] Intent-to-treat (ITT) analysis: assessment of protective efficacy against virologically confirmed, symptomatic dengue, irrespective of severity or serotype from the first injection through to the end of the 25-month active phase (see Fig. 2).

[c] According to the independent data monitoring committee (IDMC) definition. Assessment made by the investigators. Cases classified as severe based on predefined criteria and following a medical chart review (criteria extracted from SEARO 2011, WHO 1997 and WHO 2009 classifications) [89,90]. Organ dysfunction is also included in the criteria.

[d] According to the WHO 1997 classification [89]. Cases classified by statistical program. Cases without ≥2 days of fever are excluded from the WHO definition. Criteria do not include organ dysfunction.

disease *vs* 80.3% against hospitalization and 95.5% against dengue hemorrhagic fever (Table 1). Given the impact of severe disease and hospitalization for dengue on the public health systems in endemic countries, these results are pertinent from a public health perspective.

One important observation in the CYD14 trial was that efficacy increased with age [4]. In addition, in both CYD14 and CYD15 trials, prior exposure to dengue was identified as an important covariate for efficacy, which was higher in participants who were dengue seropositive at baseline compared with those who were seronegative. The increase in age presumably reflects accumulative exposure to dengue, and age may therefore represent a surrogate for seropositivity. Nevertheless, one cannot clearly discriminate whether age is a confounding factor or can have an independent impact (see Section 4.3). Moreover, our observations on the impact of serostatus at baseline come from a more limited sample size (immunogenicity

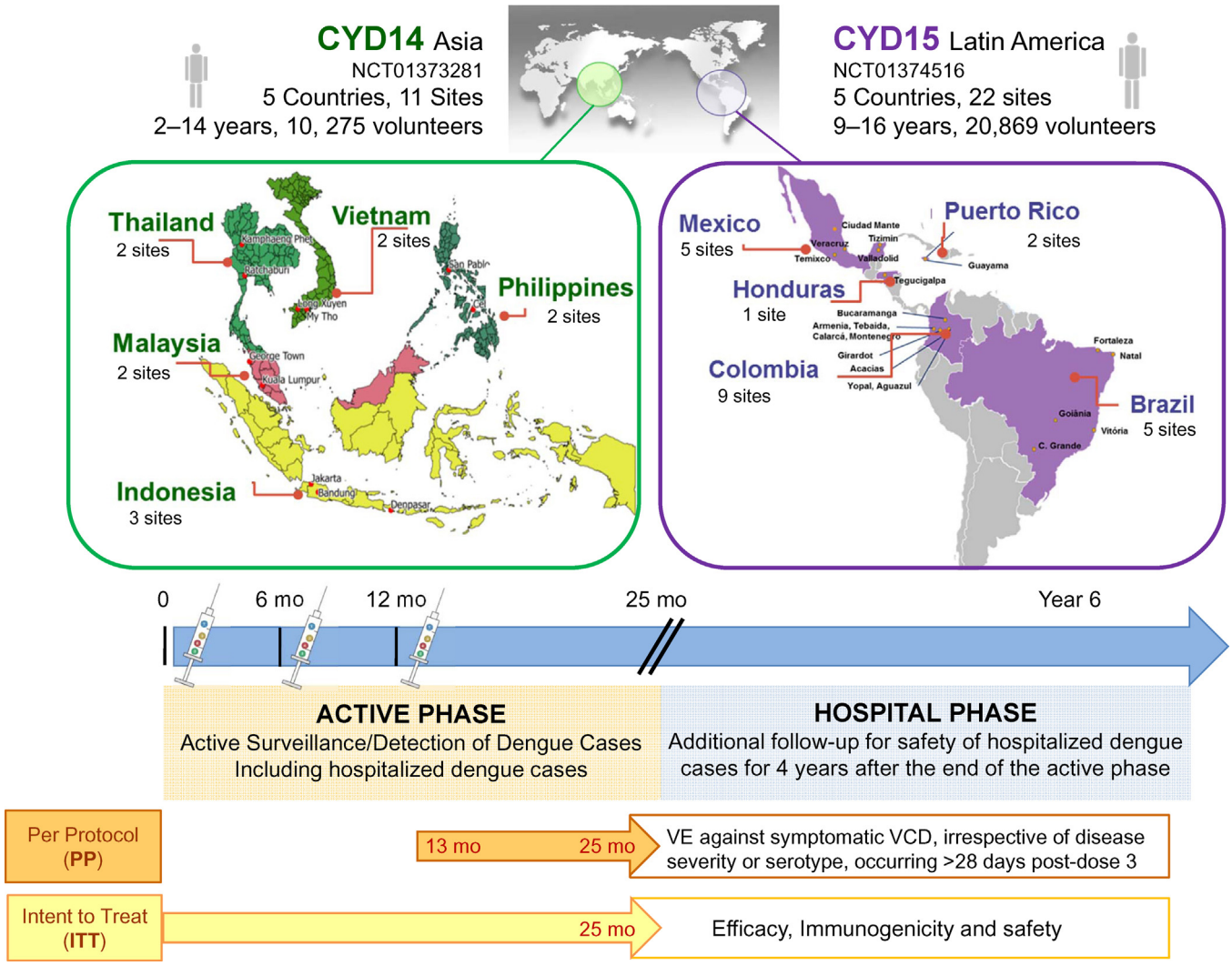

Fig. 2. Design and timelines of the two pivotal phase III efficacy studies; the results are presented in Table 1. Trials were conducted in Asia (CYD14) and Latin America (CYD15), in five countries for each. Three injections were administered 6 months apart in 2–14-year-old children in the CYD14 trial, and 9–16-year-old children and adolescents in the CYD15 trial. Per protocol (PP) and intent-to-treat (ITT) analyses were performed during the 25-month active phase, which is now completed. Both trials are halfway through the 4-year hospital phase. Abbreviations: mo, months; VCD, virologically confirmed dengue; VE, vaccine efficacy.

subset of volunteers). Meta-analysis of efficacy was also performed for the active phase of CYD14 and CYD15, and further analyses of hospital phase surveillance data (see below) prompted a focus for the pooled analysis on an age cut-point of ≥9 years, which was also the lower age of participants enrolled in CYD15 [41]. Vaccine efficacy against virologically confirmed dengue (VCD) of any severity due to all serotypes in the pooled analyses for participants aged ≥9 years in the active phase was 65.6% (95% CI: 60.7; 69.9). Pooled vaccine efficacy (VE) for seronegative individuals aged ≥9 years was 52.5% (95% CI: 5.9; 76.1). VE was also confirmed in individuals who were seropositive at baseline: 81.9 (95% CI: 67.2; 90.0). Pooled VE against hospitalization for dengue among participants aged ≥9 was 80.8% (95% CI, 70.1–87.7). In the same population, pooled VE for severe dengue, as defined according to the criteria of the independent data monitoring committee, was 93.2% (95% CI, 77.3–98.0) and the vaccine efficacy against DHF, as defined according to the WHO criteria, was 92.9% (95% CI, 76.1–97.9) [41].

In both trials, the safety profile for CYD-TDV was similar to placebo during the 25-month active phase, with no marked differences in the rates of adverse events, in agreement with previous clinical trials [3,30,31]. This safety profile linked the CYD-TV technology is a key clinical attribute when referring to the previous failure of previous empirically attenuated live dengue vaccine candidates, which evidenced substantial reactogenicity. The frequencies of serious adverse events were similar between the vaccine and control groups, and the events were consistent with medical conditions in this age group. Infections and injuries were the most commonly reported serious adverse events, but were unrelated to vaccination. No short-term or long-term safety issues potentially linked to sensitization were observed during the active phase, and no severe immediate hypersensitivity or allergic reactions were related to vaccination. Moreover, no cases of acute viscerotropic or neurotropic diseases were recorded, and no vaccine-related deaths were reported.

Beyond the active phase of surveillance during the phase 3 efficacy trials, the clinical development program for the CYD-TDV candidate vaccine includes a four-year long term follow-up (LTFU) phase, *i.e.* the Hospital Phase, starting 13 months after the third vaccine administration and ending five years after completion of the vaccination schedule to assess safety, in line with the WHO guidelines. Hospitalization for acute fever is recorded during study contacts, and by self-reporting and surveillance of the hospital networks.

During the first year of the LFTU in the multicentric Asian trial CYD14, the relative risk of hospitalization for virologically confirmed dengue in the vaccine group as compared with the control

group was 1.04 (95% confidence interval [CI], 0.52–2.19) [41]. Pre-planned analyses showed that the risk was observed in younger children, particularly in the youngest age group analyzed, 2–5 years. This shift in Relative Risk (RR) for hospitalized VCD directed us to address it separately in populations below and above 9 years; in this regard, no issue was seen in ≥9 years in the first year of long term follow up in CYD14 and in the first two years in CYD57 (follow up of the CYD23 Phase IIb trial), and similarly no issue was seen in CYD15 in pre-planned analysis ≥9 years. When considering pooled relative risks (RRs) during the first year of LTFU of CYD14, CYD15 and CYD57, RRs were 0.84 (95% CI: 0.56; 1.24), 1.58 (95% CI: 0.83; 3.02) and 0.50 (95% CI: 0.29; 0.86), for all participants, and those aged <9 and ≥9 years, respectively. Importantly, there were no clinically important differences in the frequencies of various signs and symptoms in the hospitalized participants were seen between the efficacy surveillance phase and the long-term follow-up phase in any of the studies or between the vaccine and control groups. Cumulative RRs (active phase and first year of LTFU) were: CYD14: 0.46 (95% CI: 0.32; 0.65), CYD15: 0.28 (95% CI: 0.18; 0.44) and CYD23/57: 0.66 (95% CI: 0.43; 1.02). In totality, these analyses suggested that the optimal age for intervention is from 9 years given the observed favorable clinical profile with higher efficacy for preventing VCD and an acceptable post-vaccination safety profile for individuals aged ≥9 years.

These results for both the active period and the first years of the hospital phase, which include data from 10 countries, different populations (age and ethnicity), and different dengue seasons (with different circulating serotypes and levels of endemicity), provide a consistent picture of the efficacy and safety of CYD-TDV.

4.3. Questions, investigations and next steps

Overall, the phase IIb and phase III results identified important determinants of efficacy with the CYD-TDV, in particular the varying serotype-specific efficacy, the importance of dengue baseline pre-immunity, and the potential importance of age as a surrogate for prior exposure. In addition, age could also reflect some immaturity at both the immunological and physiological levels [42,43]. These aspects have been and are still being addressed through investigations carried out in-house and/or through external collaborations [40]. Firstly, the efficacy trials showed higher protection against serotypes 3 and 4 than against serotypes 1 and 2, while similar neutralization geometric mean titer (GMT) values were observed for all four serotypes in the Vero cell-based $PRNT_{50}$ assay. Post-study investigations to understand this result included a broad array of analytical and experimental methods in four areas: host and immunity, virus, vaccine and vector [44,45]. To date, results suggest that despite the presence of key epitopes on the vaccine viruses, qualitative differences exist in responses against the different serotypes in naïve volunteers, although it is necessary to further confirm the results obtained in a larger number of sera. Ongoing, clinical, long-term follow-up and investigations should provide additional critical elements. A human challenge model, such as the one developed by the Walter Reed Army Institute of Research (WRAIR) could also provide some additional answers to some of these aspects [46]. Preliminary results from these investigations and the additional remaining questions are presented in the following points and summarized in Fig. 3:

i) Host and immunity

- What is the impact of the quantitative response against each serotype with regard to protection? Exploratory analyses using logistic regression suggest a relationship between the probability of disease and PRNT titers [44]. Some studies also suggest that levels required for protection may differ according to serotypes [47,48].
- Besides the WHO recommended $PRNT_{50}$ used in our studies [49], should alternative assays be used in replacement of, or in addition to this assay [50–52], which could bring additional information on the quality of vaccine-induced immunity? In this regard, are there qualitative differences in the responses induced by each CYD-TDV serotype? The specificity of the vaccine-induced antibodies was assessed using sera from a completed clinical trial (NCT01134263) in flavivirus-seronegative participants at baseline. Sera were depleted using virus-coated beads, and neutralization was assessed before and after depletion in a flow cytometry-based assay using U937-DC SIGN+ cells [45,52]. These experiments showed that in this dengue-naïve population, CYD-TDV vaccination elicited mostly homotypic responses against only a few serotypes (*e.g.* DENV-4), while responses against the remaining serotypes were in majority heterotypic. On the other hand, it was shown using the same assays that vaccination in dengue-primed individuals triggered a qualitatively different immune response, with no more dominance of responses against DENV-4 (see below).
- Alternatively, or in addition, could lower protection be linked to the induction of potentially enhancing responses against some particular serotypes? To address a potential sensitization/antibody-dependent enhancement (ADE) issue for lower efficacy serotypes, the balance between neutralizing antibodies and potentially enhancing ones were also assessed for each serotype in an *in vitro* assay using FcγR+ transfected CV1 cells [50,53]. It was shown in particular that vaccine-induced anti-DENV-2 and anti-DENV-1 responses were not lower than against the other two serotypes in this assay.
- Why does dengue serostatus at baseline influence subsequent vaccine efficacy? It was shown in the CYD14 and CYD15 studies that the baseline dengue serological status was an important covariate for efficacy (see above), and therefore, it was important to also characterize the type of immunity present in sera from vaccinated participants who were dengue-seropositive at baseline. It was previously observed that higher $PRNT_{50}$ responses were induced after vaccination in flavivirus seropositive than seronegative participants [28–30]. Subsequently, using depletion studies as mentioned above, it was observed in the sera from seropositive participants at baseline that a higher and broader cross-reactive response was present against all four serotypes after vaccination [45]. Cross-protection is induced during a certain period after natural wild-type dengue infection [54–59], which is further increased after secondary infection [60]. This can be linked to the induction of higher levels and quality of cross-reactive neutralizing antibodies [61–66]. Should vaccination in dengue seropositive participants at baseline act as an attenuated secondary wild-type infection, it may thus provide a better subsequent protection, which is in agreement with observations from the CYD14 and CYD15 trials.
- In addition to serostatus at baseline, could age play an additional role? Younger children may mount a less effective immune response, and/or be more susceptible to infection and severe disease because of some vascular physiological differences (ongoing development of the capillary system) [42,43,67–70]. This could also play a role in the observations made during the first year of hospital phase in the younger CYD14 population [41]. In addition, age is likely to act as a surrogate of prior exposure and seropositivity, which could explain why age-related variance in efficacy was seen in the CYD14 study.
- What is the role of cell-mediated immunity? T-cell responses induced by wild-type dengue infection can play a positive role in protection, depending on their profile and specificity (for a review see [71–74]). As mentioned previously, CYD-TDV induces T-cell

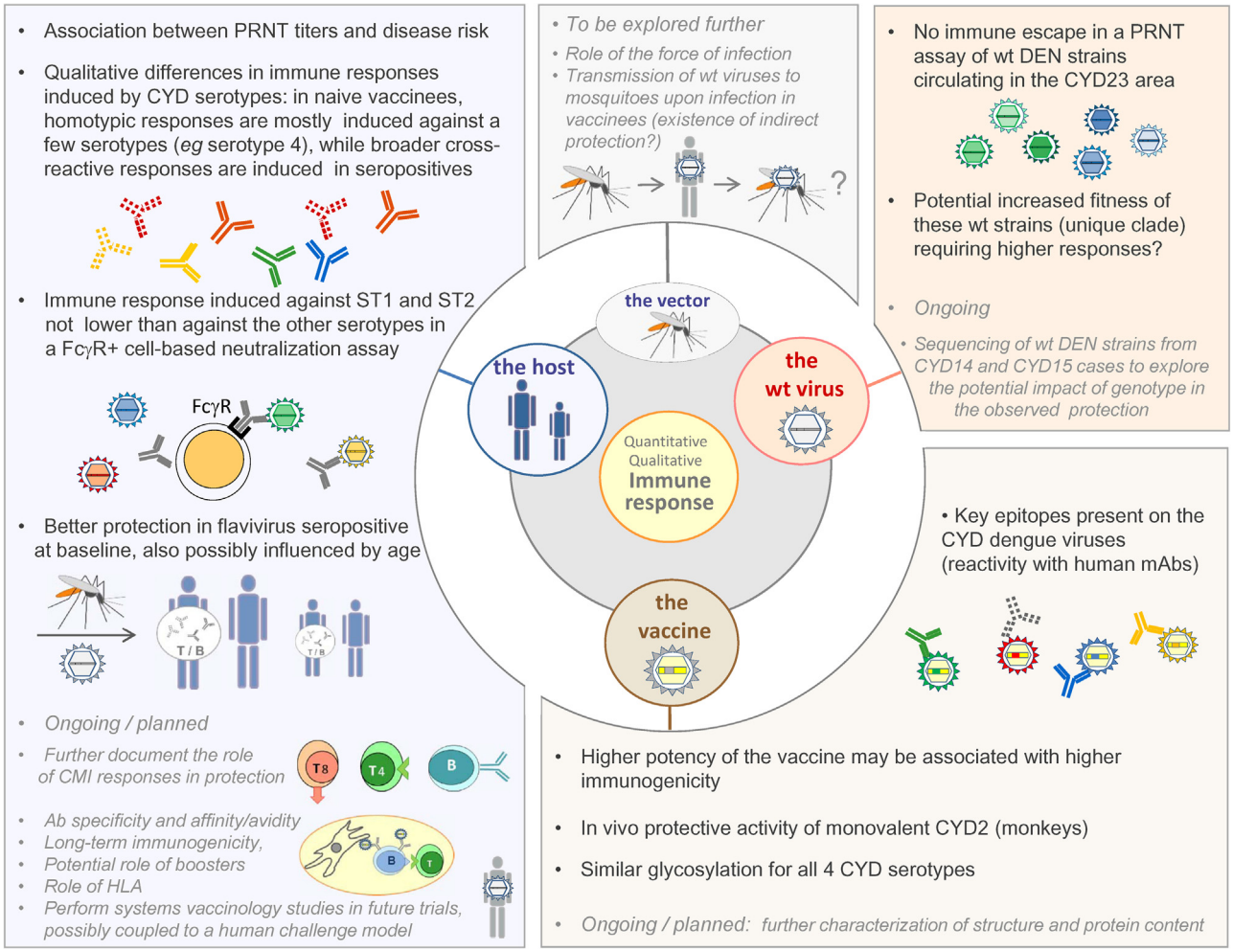

Fig. 3. Investigations carried out after the phase IIb and phase III trials (still ongoing). These investigations addressed the potential role of the vaccine, wild type virus and host immune response in the observed results. The role of the mosquito vector and force of infection have not been addressed yet. Abbreviations: Ab, antibody; CMI, cell-mediated immunity; CYD, chimeric yellow fever dengue; HLA, human leukocyte antigen; mAbs, monoclonal antibodies; PRNT, plaque-reduction neutralization test; wt, wild type.

responses against dengue structural antigens in seronegative individuals, while in seropositives, vaccination also recalls T-cell responses against dengue non-structural antigens (NS3) [35]. In this regard, what is the role of T-cell responses induced or recalled after CYD-TDV with respect to protection against infection and symptomatic disease, in both seropositive or seronegative participants? Also, might there be a role for human leukocyte antigen (HLA) or other genetic factors [75,76]?

- What is the position regarding longer term safety and efficacy? Results have been obtained during the active phase, and, as stated previously, it has been observed that transient cross-protection is induced after wild-type infection [54–60]. How can it apply to vaccination, in particular, in those who are seronegative at baseline? Data acquired during the ongoing hospital phase has already brought additional information regarding both efficacy and safety in the different populations (according to age and serostatus), and will give an indication whether or not a booster dose is required in certain populations. Ongoing long-term clinical follow-up and investigations will inform in particular on the evolution of the imbalance in VCD observed in the first year of the hospital phase in vaccinees aged <9 years. Several plausible interrelated biologic hypotheses could explain this imbalance, involving waning immunity, age/serostatus and temporal clustering of infection in vaccinees, as proposed in Hadinegoro [41]. Statistical and clinical investigations are ongoing to explore these hypotheses. The risk management plan (RMP) established upfront will also be important to assess the benefit risk profile of the vaccine over time (see below).

ii) The virus

- Could some immune escape be responsible for the lower observed efficacy despite similar responses against parental strains? It was observed that antigenic diversity between parental vaccine virus and wild-type isolates obtained in the CYD23 study—including DENV-2—did not impact neutralization in a Vero cell-based $PRNT_{50}$ assay using serum from either CYD-TDV or placebo recipients [45]. This is in agreement with a previous study showing the broad neutralization of a large range of clades and genotypes for each given serotype when using sera obtained after tetravalent vaccination [77].
- Alternatively, a potential increased fitness of these wild-type strains (unique clade [78]) could require higher serological responses for protection. Sequence analysis of viruses responsible for cases in the CYD14 and CYD15 studies is ongoing, which will bring more information on the potential link between the level of protection and circulating clades/genotypes within each serotype.

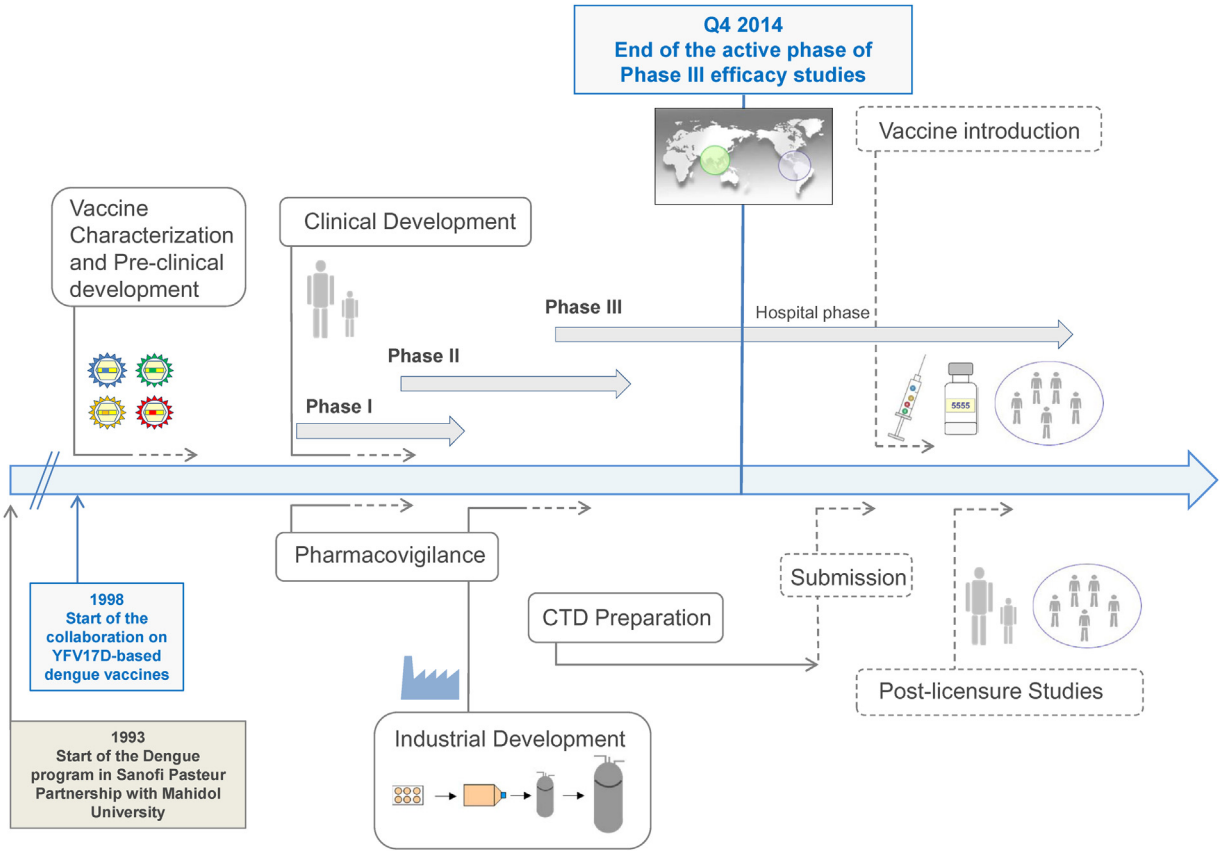

Fig. 4. Major steps of CYD-TDV development, from upstream research to vaccine introduction, covering more than 15 years. Some activities have been performed sequentially (*e.g.* initial preclinical studies followed by phase I clinical development), while some others have been launched in parallel (*e.g.* late clinical development and the building of facilities for vaccine production, as well as CTD preparation). Post-licensure phase IIIb and phase IV studies will keep documenting the effectiveness and long-term safety after vaccine introduction. Abbreviations: CTD, common technical document.

- One has also to consider that a complex interplay may exist between the nature of host dengue serostatus and the nature of the circulating clades regarding the outcome of disease [79].

iii) The vaccine

- Are there differences in the ability of each CYD-TDV serotype to be infectious *in vivo* and its ability to trigger an effective response? Beyond the initial study that showed protection in monkeys [15], it was recently demonstrated that the DENV-2 vaccine serotype (CYD-2) could confer some protection against a more stringent DENV-2 challenge in monkeys, confirming its immunogenicity *in vivo* [80].
- If serotype-specific responses vary according to serotypes, is it linked to differences in serotype-specific epitope presentation of the vaccine dengue serotypes? Despite differences in homotypic responses in naïve volunteers, it was observed using human monoclonal antibodies that key serotype-specific quaternary epitopes [81–83] were present and recognized on the corresponding DENV-1, 2, and 3 vaccine serotypes (CYD-1, -2, and -3) (monoclonal antibodies were not available for DENV-4), while cross-reactive anti-rE DI-II or DIII monoclonal antibodies recognized all four CYD dengue vaccine serotypes [45]. The use of additional monoclonal antibodies, such as those targeting the envelope dimer epitope (EDE antibody) [84], could also provide additional important information.
- Can the amount of administered vaccine viruses impact subsequent immune responses? Prior retrospective clinical data suggest a relationship between vaccine potency (the 50% cell culture infective dose; $CCID_{50}$) and neutralization [44]. It remains to be determined whether or not higher doses of live attenuated dengue vaccines could translate to incrementally higher clinical efficacy.

iv) The vector

- Given that efficacy results were consistent between the CYD14 and CYD15 trials, despite being conducted in different geographic areas and during different dengue seasons (epidemic or endemic situations), it would suggest that different forces of infection may not play a major role in the observed differences between serotypes. One point, however, which needs to be addressed further—linked with host immunity—is whether or not asymptomatic infection in those vaccinated results in sufficient viremia to allow transmission of dengue virus to uninfected mosquitoes [85]. This would impact the ability of the vaccine to provide indirect protection, and warrants further investigations.

5. Next steps: how can the benefit of the CYD-TDV be maximized?

The development and production of a safe and efficacious vaccine are the first steps toward providing protection to populations at risk from dengue. In this regard, it will be important to define the populations which will benefit the most from the CYD-TDV. In addition, a number of other challenges—including epidemiological, economic, regulatory, and logistical—must also be met to ensure the successful introduction of the vaccine in any region [2].

One of the main challenges is determining the true burden of dengue disease to judge the optimal point of intervention in a country vaccination program. Estimates of disease incidence and burden that rely solely on the number of reported cases will inevitably underestimate the magnitude of the problem. Improved surveillance systems would improve the quantification of the medical value of dengue vaccination programs. The epidemiology of dengue varies considerably, both geographically and temporally, which may impact the way the vaccine would be used in each country. Computational modeling will play a substantial role collating multiple parameters with which to assess the impact of vaccination on disease burden at a population level and therefore assist national organizations identify the optimal programs and catch-up cohorts for the maximal public health benefit. In this regard, parameters related to vaccine efficacy and levels of dengue transmission have been estimated using data collected during the phase III efficacy studies, and several vaccination programs including routine vaccination at different ages completed or not by large catch-up campaigns have been investigated, supporting the benefit which could be afforded by the vaccine.

Vaccination against dengue should also be considered as part of a wider, integrated strategy with community involvement, surveillance, case management, vector and outbreak control. Governments will need to anticipate budget needs for routine dengue vaccination, catch-up programs, consumables, infrastructure, training, and surveillance. Alternative funding mechanisms may be needed to finance vaccination programs in some countries located in endemic zones.

The initial introduction of dengue vaccination will be accompanied by phase IV studies that should be planned in collaboration with national authorities, and will serve to reinforce the medical value (including effectiveness and safety), impact (*e.g.* indirect effect), and feasibility of vaccination. In particular, a Pharmacovigilance Risk Management Plan (RMP) has been established to present the strategy that Sanofi Pasteur will deploy once the CYD dengue vaccine is licensed and marketed in order to monitor and evaluate adverse events associated with the use of CYD dengue vaccine, and evaluate vaccine effectiveness in real-life use. While no important risks have been identified for CYD dengue vaccine during its clinical development in the target population, this plan has been designed taking into consideration all clinical data including the observations outside the targeted age indication (below 9 years of age). This action plan includes Post-Authorization Safety Studies (PASS) and Post-Authorization Effectiveness Studies (PAES) in addition to the continuous follow-up of efficacy studies (up to 5 years after last dose injection).

6. Conclusions

The data from the active phase of the two, pivotal, phase III efficacy studies demonstrated that the CYD-TDV is efficacious with a satisfactory safety profile, out to 25 months after the first injection, and suggest that this vaccine can contribute to meet the WHO objectives to reduce by 2020 the dengue mortality by 50% and morbidity by 25%. The end of these active phases closes more than a decade of development of the CYD-TDV, although further follow up, activities and efforts remain to deepen our understanding of the vaccine, and also to prepare for the introduction and use of the Sanofi Pasteur dengue vaccine (Fig. 4). The observations made in the first years of the hospital phase of the efficacy studies have contributed to better define the target population (*i.e.* from 9 years of age), in order to drive the optimal public health impact through prophylaxis, and the development of tools and actions taken to reach that goal. Dengue is a complex disease, and both short-term and long-term safety, and efficacy will have to be considered further to assess the overall benefit of the vaccine for human health. This is ongoing and will continue to be addressed by long-term follow-up and future post-licensure studies, with results available in the coming years. Our predefined risk management plan is designed to confirm our safety profile in a real-world use setting after vaccine implementation.

In conclusion, the development of a dengue vaccine, similar to other recent vaccine successes against human papillomavirus and pneumococcal disease, requires a long standing and continuous effort from private and public organizations. In this regard, we have in the past 22 years benefited from multiple external collaborations across five continents. Since the very beginning, these have been critical in developing a vaccine that might eventually bring a solution to the still growing and worldwide problem presented by dengue [86,87]. Moving forward, there is a need for the development of new models and collaborations in order to better decipher the immunological mechanisms triggered by CYD-TDV. One such example being the use of translational science models, and in particular systems vaccinology approaches and tools [88].

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References

[1] 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.
[2] 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.
[3] Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, 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.
[4] Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, 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.
[5] Villar L, Dayan GH, Arredondo-Garcia JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med 2015;372(2):113–23.
[6] Thomas SJ. Preventing dengue – is the possibility now a reality? N Engl J Med 2015;372(2):172–3.
[7] Barban V, Girerd Y, Aguirre M, Gulia S, Petiard F, Riou P, et al. High stability of yellow fever 17D-204 vaccine: a 12-year restrospective analysis of large-scale production. Vaccine 2007;25(15):2941–50.
[8] Pugachev KV, Guirakhoo F, Ocran SW, Mitchell F, Parsons M, Penal C, et al. High fidelity of yellow fever virus RNA polymerase. J Virol 2004;78(2):1032–8.
[9] Mantel N, Girerd Y, Geny C, Bernard I, Pontvianne J, Lang J, et al. Genetic stability of a dengue vaccine based on chimeric yellow fever/dengue viruses. Vaccine 2011;29(38):6629–35.
[10] Dubayle J, Vialle S, Schneider D, Pontvianne J, Mantel NOA, et al. Site-specific characterization of envelope protein N-glycosylation on Sanofi Pasteur's tetravalent CYD dengue vaccine. Vaccine 2015;33(11):1360–8.
[11] Brandler S, Brown N, Ermak TH, Mitchell F, Parsons M, Zhang Z, et al. 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.
[12] Deauvieau F, Sanchez V, Balas C, Kennel A, DE Montfort A, Lang J, et al. 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.

[13] Balas C, Kennel A, Deauvieau F, Sodoyer R, Arnaud-Barbe N, Lang J, et al. 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):103–8.
[14] Guirakhoo F, Weltzin R, Chambers TJ, Zhang ZX, Soike K, Ratterree M, et al. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. J Virol 2000;74(12):5477–85.
[15] Guirakhoo F, Pugachev K, Zhang Z, Myers G, Levenbook I, Draper K, et al. Safety and efficacy of chimeric yellow Fever-dengue virus tetravalent vaccine formulations in nonhuman primates. J Virol 2004;78(9):4761–75.
[16] Guy B, Barban V, Mantel N, Aguirre M, Gulia S, Pontvianne J, et al. Evaluation of interferences between dengue vaccine serotypes in a monkey model. Am J Trop Med Hyg 2009;80(2):302–11.
[17] Ravel G, Guy B, Mantel N, Lang J, Gould S. Sanofi Pasteur live attenuated, tetravalent dengue vaccine toxicity, biodistribution and shedding study in cynomolgus monkeys. Abstract 630. Am J Trop Med Hyg 2012;87(5 Suppl 1):150–226.
[18] Higgs S, Vanlandingham DL, Klingler KA, McElroy KL, McGee CE, Harrington L, et al. 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.
[19] Kazimirova M, Mantel N, Raynaud S, Slovak M, Ustanikova K, Lang J, et al. Evaluation of chimeric yellow fever 17D/dengue viral replication in ticks. Vector Borne Zoonotic Dis 2012;12(11):979–85.
[20] Monath TP, Myers GA, Beck RA, Knauber M, Scappaticci K, Pullano T, 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.
[21] McGee CE, Lewis MG, Claire MS, Wagner W, Lang J, Guy B, et al. 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):693–7.
[22] McGee CE, Tsetsarkin K, Vanlandingham DL, McElroy KL, Lang J, Guy B, et al. 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.
[23] McGee CE, Tsetsarkin KA, Guy B, Lang J, Plante K, Vanlandingham DL, et al. Stability of yellow fever virus under recombinatory pressure as compared with chikungunya virus. PLOS ONE 2011;6(8):e23247.
[24] Lefeuvre A. Caractérisation des interactions entre les cellules hépatiques et les souches sauvage et vaccinale du virus de la fièvre jaune: application à l'étude de virus fièvre jaune chimériques utilisés pour le développement d'un vaccin contre la dengue. In: Diplôme de Doctorat 2005; Université Claude Bernard Lyon 1, No 129. 2005.
[25] Guy B, Chanthavanich P, Gimenez S, Sirivichayakul C, Sabchareon A, Begue S, et al. Evaluation by flow cytometry of antibody-dependent enhancement (ADE) of dengue infection by sera from Thai children immunized with a live-attenuated tetravalent dengue vaccine. Vaccine 2004;22(27–28):3563–74.
[26] 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):370–7.
[27] Poo J, Galan F, Forrat R, Zambrano B, Lang J, Dayan GH. 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.
[28] Capeding RZ, Luna IA, Bomasang E, Lupisan S, Lang J, Forrat R, et al. 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.
[29] 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.
[30] Lanata CF, Andrade T, Gil AI, Terrones C, Valladolid O, Zambrano B, et al. 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.
[31] Leo YS, Wilder-Smith A, Archuleta S, Shek LP, Chong CY, Leong HN, 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.
[32] Hss AS, Koh MT, Tan KK, Chan LG, Zhou L, Bouckenooghe A, et al. 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.
[33] Guirakhoo F, Kitchener S, Morrison D, Forrat R, McCarthy K, Nichols R, 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):60–7.
[34] Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine 2008;26(45):5712–21.
[35] Harenberg A, Begue S, Mamessier A, Gimenez-Fourage S, Ching Seah C, Wei Liang 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.
[36] Dayan G, Galán-Herrera G, Forrat R, Zambrano B, Bouckenooghe A, Harenberg A, et al. Assessment of bivalent and tetravalent dengue vaccine formulations in flavivirus-naïve adults in Mexico. Hum Vaccin Immunother 2014;10(10):2853–63.
[37] European Medicines Agency. Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. EMA/CHMP/VWP/141697/2009; 2010.
[38] Monath TP, Seligman SJ, Robertson JS, Guy B, Hayes EB, Condit RC, et al. Live virus vaccines based on a yellow fever vaccine backbone: standardized template with key considerations for a risk/benefit assessment. Vaccine 2015;33(1):62–72.
[39] World Health Organization. Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). Geneva: World Health Organization, WHO Expert Committee on Biological Standardization; WHO Technical Report Series, No. 979 Annex 2 (Replacement of Annex 1 of WHO Technical Report Series, No. 932 adopted by ECBS in 2011. 2011. Available at: http://www.who.int/biologicals/areas/vaccines/TRS_979_Annex_2pdf?ua=1).
[40] Jackson N. Sanofi Pasteur dengue vaccine. In: The American Society of Tropical Medicine and Hygiene 63rd Annual Meeting, November 3rd Symposium 2014. 2014.
[41] Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373:1195–206.
[42] Gamble J, Bethell D, Day NP, Loc PP, Phu NH, Gartside IB, et al. Age-related changes in microvascular permeability: a significant factor in the susceptibility of children to shock? Clin Sci (Lond) 2000;98(2):211–6.
[43] Hanna-Wakim R, Yasukawa LL, Sung P, Fang M, Sullivan B, Rinki M, et al. Age-related increase in the frequency of CD4(+) T cells that produce interferon-gamma in response to staphylococcal enterotoxin B during childhood. J Infect Dis 2009;200(12):1921–7.
[44] Jackson N, Boaz M, Hu B, Langevin E, Byers A, Baric R, et al. Investigations of the observed efficacy of the CYD tetravalent dengue vaccine in the phase 2b trial in Ratchaburi, Thailand. Abstract 576. Am J Trop Med Hyg 2014;91(5 Suppl 1):149–228.
[45] Guy B, Boaz M, Byers A, Saulnier A, de Silva A, Henein S, et al. Assessment of the qualitative immune response induced by the CYD tetravalent dengue vaccine in human volunteers. Abstract 575. Am J Trop Med Hyg 2014;91(5 Suppl 1):149–228.
[46] Thomas SJ. Dengue human infection model: re-establishing a tool for understanding dengue immunology and advancing vaccine development. Hum Vaccin Immunother 2013;9(7):1587–90.
[47] Endy TP, Nisalak A, Chunsuttitwat S, Vaughn DW, Green S, Ennis FA, et al. 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.
[48] Buddhari D, Aldstadt J, Endy TP, Srikiatkhachorn A, Thaisomboonsuk B, Klungthong C, et al. Dengue virus neutralizing antibody levels associated with protection from infection in thai cluster studies. PLoS Negl Trop Dis 2014;8(10):e3230.
[49] Timiryasova TM, Bonaparte MI, Luo P, Zedar R, Hu BT, Hildreth SW. Optimization and validation of a plaque reduction neutralization test for the detection of neutralizing antibodies to four serotypes of dengue virus used in support of dengue vaccine development. Am J Trop Med Hyg 2013;88(5):962–70.
[50] Rodrigo WW, Alcena DC, Rose RC, Jin X, Schlesinger JJ. An automated Dengue virus microneutralization plaque assay performed in human Fc{gamma} receptor-expressing CV-1 cells. Am J Trop Med Hyg 2009;80(1):61–5.
[51] Putnak JR, de la Barrera R, Burgess T, Pardo J, Dessy F, Gheysen D, et al. Comparative evaluation of three assays for measurement of dengue virus neutralizing antibodies. Am J Trop Med Hyg 2008;79(1):115–22.
[52] de Alwis R, de Silva AM. Measuring antibody neutralization of dengue virus (DENV) using a flow cytometry-based technique. Methods Mol Biol 2014;1138:27–39.
[53] Byers A, Broder R, Moser J, Boaz M, Warren W, Jackson N, et al. Serotype-specific dengue neutralizing antibody responses in FcgR expressing CV1 cells. Abstract 833. Am J Trop Med Hyg 2014;91(5 Suppl 1):228–303.
[54] Sabin AB. Research on dengue during World War II. Am J Trop Med Hyg 1952;1(1):30–50.
[55] Snow GE, Haaland B, Ooi EE, Gubler DJ. Review article: research on dengue during World War II revisited. Am J Trop Med Hyg 2014;91(6):1203–17.
[56] Balmaseda A, Standish K, Mercado JC, Matute JC, Tellez Y, Saborio S, et al. Trends in patterns of dengue transmission over 4 years in a pediatric cohort study in Nicaragua. J Infect Dis 2010;201(1):5–14.
[57] Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, et al. Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis 2011;5(3):e975.
[58] Anderson KB, Gibbons RV, Cummings DA, Nisalak A, Green S, Libraty DH, 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):360–8.
[59] Montoya M, Gresh L, Mercado JC, Williams KL, Vargas MJ, Gutierrez G, 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.
[60] Olkowski S, Forshey BM, Morrison AC, Rocha C, Vilcarromero S, Halsey ES, et al. Reduced risk of disease during postsecondary dengue virus infections. J Infect Dis 2013;208(6):1026–33.
[61] Mathew A, West K, Kalayanarooj S, Gibbons RV, Srikiatkhachorn A, Green S, et al. B-cell responses during primary and secondary dengue virus infections in humans. J Infect Dis 2011;204(10):1514–22.
[62] Lai CY, Williams KL, Wu YC, Knight S, Balmaseda A, Harris E, et al. Analysis of cross-reactive antibodies recognizing the fusion loop of envelope protein and correlation with neutralizing antibody titers in Nicaraguan dengue cases. PLoS Negl Trop Dis 2013;7(9):e2451.
[63] Tsai WY, Lai CY, Wu YC, Lin HE, Edwards C, Jumnainsong A, et al. High-avidity and potently neutralizing cross-reactive human monoclonal antibodies derived from secondary dengue virus infection. J Virol 2013;87(23):12562–75.
[64] Patel B, Sapparapu G, Crowe J, Baric R, de Silva A. Characterization of neutralizing antibody responses following natural secondary dengue virus infections. Abstract 626. Am J Trop Med Hyg 2014;91(5 Suppl 1):149–228.
[65] Zompi S, Montoya M, Pohl MO, Balmaseda A, Harris E. Dominant cross-reactive B cell response during secondary acute dengue virus infection in humans. PLoS Negl Trop Dis 2012;6(3):e1568.
[66] Corbett KS, Katzelnick L, Tissera H, Amerasinghe A, de Silva AD, de Silva AM. Preexisting neutralizing antibody responses distinguish clinically inapparent and apparent dengue virus infections in a Sri Lankan pediatric cohort. J Infect Dis 2015;211(4):590–9.
[67] Anders KL, Nguyet NM, Chau NV, Hung NT, Thuy TT, Lien le B, et al. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg 2011;84(1):127–34.
[68] Guzman MG, Kouri G, Bravo J, Valdes L, Vazquez S, Halstead SB. Effect of age on outcome of secondary dengue 2 infections. Int J Infect Dis 2002;6(2):118–24.
[69] Hammond SN, Balmaseda A, Perez L, Tellez Y, Saborio SI, Mercado JC, 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.
[70] Ooi EE, Goh KT, Chee Wang DN. Effect of increasing age on the trend of dengue and dengue hemorrhagic fever in Singapore. Int J Infect Dis 2003;7(3):231–2.
[71] Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol 2011;11(8):532–43.
[72] Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, et al. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. Proc Natl Acad Sci U S A 2013;110(22):E2046–53.
[73] Zellweger RM, Eddy WE, Tang WW, Miller R, Shresta S. CD8+ T cells prevent antigen-induced antibody-dependent enhancement of dengue disease in mice. J Immunol 2014;193(8):4117–24.
[74] Zellweger RM, Miller R, Eddy WE, White LJ, Johnston RE, Shresta S. Role of humoral versus cellular responses induced by a protective dengue vaccine candidate. PLoS Pathog 2013;9(10):e1003723.
[75] Stephens HA. HLA and other gene associations with dengue disease severity. Curr Top Microbiol Immunol 2010;338:99–114.
[76] Whitehorn J, Chau TN, Nguyet NM, Kien DT, Quyen NT, Trung DT, et al. Genetic variants of MICB and PLCE1 and associations with non-severe dengue. PLOS ONE 2013;8(3):e59067.
[77] Barban V, Munoz-Jordan JL, Santiago GA, Mantel N, Girerd Y, Gulia S, et al. 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):91–8.
[78] Gubler DJ, Ooi EE, Vasudevan S, editors. Dengue and Dengue Hemorrhagic Fever. 2nd Edition. Wallingford, United Kingdom: CABI Publishing; 2014. ISBN-10: 1845939646.
[79] OhAinle M, Balmaseda A, Macalalad AR, Tellez Y, Zody MC, Saborio S, et al. Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. Sci Transl Med 2011;3(114), 114ra128.
[80] Barban V, Mantel N, Harenberg A, De Montfort A, Charnay C, Jackson N, et al. Protective immunity induced against DENV-2 intravenous challenge in non-human primates immunized with CYD vaccine clinical lots. Abstract LB-3233. In: The American Society of Tropical Medicine and Hygiene 63rd Annual Meeting 2014. 2014. http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=52126352-c6f3-46f9-9c1f-c18e06fe67c4&cKey=d49492d8-dcd8-4bab-b76b-a1e374fbb4c5&mKey=%7b52AE2426-7F12-4D2B-9404-C0D0B5A8EB5A%7d.
[81] de Alwis R, Smith SA, Olivarez NP, Messer WB, Huynh JP, Wahala WM, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. Proc Natl Acad Sci U S A 2012;109(19):7439–44.
[82] Smith SA, de Alwis AR, Kose N, Harris E, Ibarra KD, Kahle KM, et al. The potent and broadly neutralizing human dengue virus-specific monoclonal antibody 1C19 reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein. mBio 2013;4(6), e00873-913.
[83] Smith SA, de Alwis AR, Kose N, Jadi RS, de Silva AM, Crowe Jr JE. Isolation of dengue virus-specific memory B cells with live virus antigen from human subjects following natural infection reveals the presence of diverse novel functional groups of antibody clones. J Virol 2014;88(21):12233–41.
[84] Dejnirattisai W, Wongwiwat W, Supasa S, Zhang X, Dai X, Rouvinsky A, et al. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. Nat Immunol 2015;16(2):170–7.
[85] Nguyet MN, Duong TH, Trung VT, Nguyen TH, Tran CN, Long VT, et al. Host and viral features of human dengue cases shape the population of infected and infectious Aedes aegypti mosquitoes. Proc Natl Acad Sci U S A 2013;110(22):9072–7.
[86] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496(7446):504–7.
[87] Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. Nat Rev Microbiol 2010;8(12 Suppl):S7–16.
[88] Pulendran B, Li S, Nakaya HI. Systems vaccinology. Immunity 2010;33(4):516–29.
[89] Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue viral infections. Postgrad Med J 2004;80:588–601.
[90] WHO. Dengue guidelines for diagnosis, treatment, prevention and control; 2009. http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf.