Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?

Questions Raised by the Development and Implementation of Dengue Vaccines: Example of the Sanofi Pasteur Tetravalent Dengue Vaccine

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Dengue is a still-growing public health concern in many tropical and subtropical regions of the world. The development and implementation of an effective dengue vaccine in these regions is a high priority. This insight focuses on the expected characteristics of a safe and efficacious vaccine, referring to the clinical experience obtained during the development of the first tetravalent dengue vaccine from Sanofi Pasteur, now licensed in several endemic countries. Safety and efficacy data from both short- and long-term follow-up of large-scale efficacy studies will be discussed, as well as the next steps following vaccine introduction.

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GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed
Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

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WHAT SHOULD BE THE CHARACTERISTICS OF A SAFE AND EFFICACIOUS VACCINE?

Dengue—a mosquito-borne disease caused by one of four dengue virus (DENV) serotypes (DENV-1 to 4)—is a growing public health problem associated with substantial morbidity, as well as significant social and economic costs in the tropic and subtropic regions of the world. The development and implementation of an effective dengue vaccine is a high priority for countries where the disease is endemic. This overview addresses two questions related to the development and implementation of dengue vaccines. The first question is, what should be the characteristics of a safe and efficacious vaccine? The second, how concerned should we be about putative enhanced disease after vaccination? To answer these questions, one can refer to the experience obtained during the clinical development of the recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine from Sanofi Pasteur, referred to in the following sections as CYD-TDV (chimeric yellow fever dengue-tetravalent dengue vaccine). It is the first DENV to market, and is already licensed as Dengvaxia for use in individuals aged 9–45 years in a number of dengue-endemic countries.

Dengue is a still-growing public health concern in many tropical and subtropical regions of the world (Bhatt et al. 2013). About two-thirds or more of dengue infections are asymptomatic. In the remainder of cases, symptoms may vary from a mild flu-like syndrome to "classical" dengue fever, which may progress in a few percent of cases to a severe life-threatening disease. Although the underlying mechanisms are not totally understood, severe dengue is proposed to be mainly the consequence of an immunopathological reaction that is initiated early in the course of infection. This reaction would involve innate responses (particularly in the case of primary infection), with the additional contribution of adaptive responses (particularly in the case of secondary infection). The latter is a well-hypothesized risk factor for severity (for a review, see Guzman et al. 2010; Rothman 2011).

Both innate and adaptive immune mechanisms are contributing factors to direct responses in a protective or nonprotective direction; measuring these responses (e.g., neutralizing antibodies, cytokines, cellular effectors) helps better identify the type of immunity linked to protection (for instance, see Fink et al. 2007; Simmons et al. 2007; Long et al. 2009). Although innate response can influence subsequent adaptive responses, the latter may also contribute through memory cells to rapidly trigger certain innate mechanisms, including cytokine production on reexposure to a new dengue virus in addition to specific effector responses.

Overall, a protective vaccine should preferably induce (1) at the innate level, moderate levels of proinflammatory cytokines and chemokines (e.g., interleukins [IL]-6, IL-8, or tumor necrosis factor α [TNF-α]), type I interferon (IFN) responses, and chemokines linking innate and adaptive immune responses; (2) at the B-cell level, a multitypic antibody response with specific and cross-reactive neutralizing antibodies at high-enough levels comparable to those induced by a second wild-type dengue infection; (3) at the T-cell level, serotype-specific CD4 and/or CD8 responses of the T helper (T_H)1/cytotoxic T (T_C)1 type directed against both structural and nonstructural antigens, with no overt inflammatory profile (IFN-γ > TNF-α) (Guy and Jackson 2016). The levels of the cytokines released and their relative kinetic profiles of appearance influences outcome. For example, some factors involved in innate or T-cell responses may play a "positive" role at the onset of disease or in terminating inflammatory reactions, but may well exacerbate "detrimental" effects as the disease progresses (Rothman 2011). These points are shown in Figure 1.

Ideally, vaccines should induce both potent humoral (neutralizing antibodies) and cellular (T_H1/T_C lymphocyte) protective immunity. Live attenuated vaccines could be optimal in this respect. To stir up an immune response, attenuated strains must be able to replicate well in vivo (ideally, simultaneously against all four serotypes), but with little systemic replication to avoid the induction of dengue-associated symptoms of fever, headache, myalgia/

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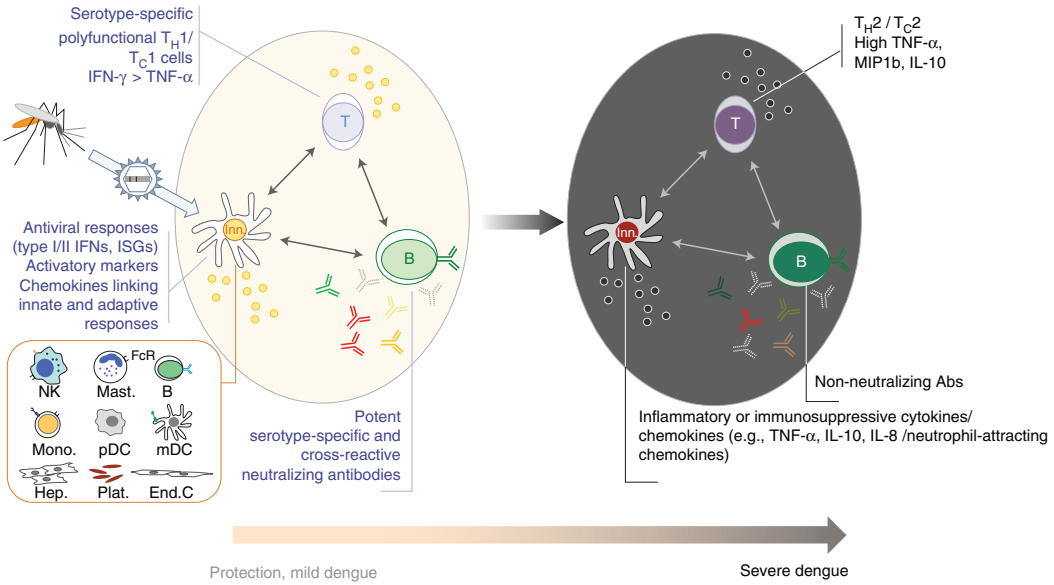

Figure 1. Immune responses to dengue infection: stay away from the dark side. The three arms of the immune system cooperate to induce protective immunity, but the dengue virus (DENV) may turn a friendly immune system in an overreactive or inappropriate one with deleterious consequences (see also Guy et al. 2016). Innate responses represent the first line of defense against dengue virus and involve, in particular, monocytes, macrophages, and myeloid dendritic cells (Ho et al. 2001; Kyle et al. 2007; Cerny et al. 2014), the latter being critical antigen-presenting cells in the initiation of primary responses. Dendritic cells in the skin are among the primary cells that are infected after inoculation from a mosquito bite, and subsequently migrate to the draining lymph nodes (Schmid and Harris 2014) where they interact with T cells. DENV can also infect and/or stimulate other types of antigen-presenting cells such as B cells (Lin et al. 2002; Wang et al. 2006) and plasmacytoid dendritic cells (Palmer et al. 2005), the latter significantly contributing to the antiviral type I interferon (IFN) response. Mechanisms that are found to be linked to the activity of several nonstructural dengue proteins can counteract antiviral type I IFN responses (for review, see Green et al. 2014) and impact the role of other cells such as mast cells, endothelial cells, and platelets (King et al. 2002; Medin et al. 2005; Souza et al. 2009; Vervaeke et al. 2015). Overall, the activation of innate system drives an antiviral response and triggers the expression of a wide array of proinflammatory and anti-inflammatory cytokines and chemokines, whose levels and kinetic profiles subsequently modulate the adaptive response and influence the outcome of the disease. Adaptive responses play an even more important role in second or subsequent dengue infection(s) caused by a homologous or heterologous serotype. Preexisting cellular responses (T helper [T_H] and cytotoxic T [T_C] cells) may limit the extent of infection by rapidly killing infected cells and secreting large quantities of inflammatory cytokines (for review, see Mathew et al. 2011; Rothman 2011; Weiskopf et al. 2013; Zellweger et al. 2013, 2014), as well as through antibody-dependent cellular cytotoxicity (Laoprasopwattana et al. 2007). However, although both humoral and cellular immune response play a role in disease protection, they have also been linked to immunopathology (dark side of the immune force, *right* side of the figure), acting detrimentally under certain conditions. In particular, primary infection may sensitize individuals to more severe second infection caused by a different serotype. This phenomenon has been hypothesized to be linked, in particular, to non-neutralizing-enhancing antibodies facilitating virus uptake through Fc receptors. This mechanism is known as antibody-dependent enhancement (ADE) and/or a detrimental inflammatory or biased T-cell response (for review, see Guzman et al. 2010; Rothman 2011). TNF, Tumor necrosis factor; IL, interleukin; ISG, interferon-stimulated gene; NK, natural killer; Mast, mast cells; B, B cells (involved here as antigen-presenting cells); Inn, innate; Mono, monocytes; pDC, plasmacytoid dendritic cell; mDC, myeloid dendritic cell; Hep, hepatocyte; plat., platelet; End. C, endothelial cell.

arthralgia, and associated modifications of biological parameters, such as blood formula and levels of some liver enzymes. In addition, they must be genetically stable for the critical attenuation mutations because any reversion, either during batch manufacture of the vaccine or after administration, may adversely affect safety. Most importantly, the strains must be incapable of transmission by mosquitoes because this may facilitate an evolutionary change toward virulence. Such transmission is unlikely if viremia is low, but mutations restricting replication in the mosquito host are also desirable (Guy et al. 2015a).

CYD-TDV (chimeric yellow fever dengue-tetravalent dengue vaccine) is composed of four recombinant vaccine viruses built on a yellow fever 17D vaccine backbone (for a review, see Guy et al. 2015a, 2016). First, regarding in vitro infectivity and immunogenicity (innate responses), all vaccine viruses (CYD-1 to 4) show growth kinetics similar to their parent viruses (wild-type DENV and YF-17D) in human monocyte-derived dendritic cells (mDCs) (Brandler et al 2005). In addition, vaccine virus infection of mDCs induces maturation and a controlled innate response, as seen by limited inflammatory cytokine production and significant expression of antiviral type I IFN and chemokines linking innate and adaptive responses (Deauvieau et al. 2007; Balas et al. 2011). The four serotypes also grow to significantly lower titers than YF-17D virus in the human hepatic cell lines THLE-3 and HepG2 (Brandler et al. 2005).

Second, moving to adaptive immunity, the following paragraphs will address first responses in subjects serologically naïve toward dengue before vaccination (referred to as "seronegatives"), and then in subjects preimmune against at least one serotype before vaccination (referred to as "seropositives"). Clinical studies have shown that three doses of CYD-TDV in seronegative individuals induce neutralizing antibodies (seroconversion) against all four serotypes, as measured by the plaque-reduction neutralization test (PRNT). The PRNT, a quantitative functional antibody assay, is considered the "gold standard" for characterizing and quantifying levels of antidengue-neutralizing antibody (for review, see Guy et al. 2015a, 2016). Exploring further qualitative aspects, recent investigations suggest that the three-dose regimen in seronegative individuals induces predominantly a homotypic-type response dominated by one serotype (usually serotype 4), whereas responses against the other serotypes are largely cross-reactive. Homotypic-type responses can nevertheless still be observed against some of the other serotypes, but are variable across individuals (Henein et al. 2017).

At the T-cell level, CYD-TDV induces serotype-specific T_H1 and/or T_C1 responses to structural antigens from all four dengue serotypes, as measured by T_H1/T_H2 cytokine expression on viral stimulation. The serotype specificity of the T-cell responses has been confirmed with the bivalent dengue vaccine (Guy et al. 2008; Harenberg et al. 2013; Dayan et al. 2014). Additionally, CYD-TDV leads to the generation of $CD8/T_C1$ responses directed against the YF-17D NS3 antigen (Guy et al. 2008; Harenberg et al. 2013; Dayan et al. 2014). Overall, the three-dose vaccination schedule in seronegative individuals, while inducing seroconversion in the majority of vaccinees, may partially mimic an attenuated or subclinical primary infection, with different features given the tetravalent nature of CYD-TDV.

In seropositive individuals, vaccine-induced PRNT responses are higher (Guy et al. 2009; Qiao et al. 2011; Capeding et al. 2014; Villar et al. 2015) and broader than in seronegative individuals, being mostly cross-reactive against all four serotypes in the former population. Vaccination boosts underlying wild-type-induced preimmunity through recall responses by memory cells. Recent findings indicate that this response is specific to the first infecting serotype and cross-reactive (Henein et al. 2017). Importantly, a broader cellular response has also been detected in seropositive individuals, including boosting of antidengue NS3 CD8 responses (Harenberg et al. 2013). Overall, vaccination in seropositive individuals appears to mimic an attenuated and subclinical heterotypic (multivalent) second wild-type-like infection.

The active phase of the two large-scale CYD-TDV efficacy trials (CYD14 and CYD15), is now completed, providing efficacy and safety results from the first 25 months following the initial vaccination (Capeding et al. 2014; Villar et al. 2015). A pooled analysis of these two studies was performed for the active phase in participants aged ≥9 years, the indicated target population for the vaccine (see below) (Hadinegoro et al. 2015). Vaccine efficacy during the 25-month active phase in the pooled analysis of the trials was 65.6% (95% confidence interval [CI], 60.7–69.9) against virologically confirmed dengue (VCD) of any severity owing to all serotypes, 80.8% (95% CI, 70.1–87.7) against hospitalization, 93.2% (95% CI, 77.3–98.0) against severe dengue as defined according to the criteria of the independent data monitoring committee, and 92.9% (95% CI, 76.1–97.9) against dengue hemorrhagic fever (DHF) as defined according to the World Health Organization (WHO) criteria. Vaccine efficacy varied by serotype, both trials showing higher efficacy rates against DENV-3 and DENV-4 than against DENV-1 and DENV-2, as had been observed in a previous phase IIb trial in Thailand (Sabchareon et al. 2012). Both phase III trials also identified important covariates for efficacy: It increased with age in the CYD14 trial (Capeding et al. 2014), and higher protection was seen in seropositive than in seronegative participants. The increase in age reflecting accumulative exposure to dengue may therefore serve as a surrogate of seropositivity, but may also play an independent role, as discussed below.

HOW CONCERNED SHOULD WE BE ABOUT PUTATIVE ENHANCED DISEASE AFTER VACCINATION?

Beyond the active phase, the clinical development program for CYD-TDV includes a 4-year long-term follow-up, monitoring hospitalized cases from the phase IIb and two phase III trials, that is, the hospital phase, starting 13 months after the third vaccination and ending 5 years after completion of the vaccination schedule to assess safety, in line with the WHO guidelines. During the first year of the long-term follow-up in the CYD14 trial (year 3 of the study), a higher incidence of hospitalized VCD was observed in the younger population (Hadinegoro et al. 2015). The risk was particularly high in the youngest age group, 2–5 years. There was no issue seen in children aged ≥9 years in CYD14 and CYD57 (follow-up of the CYD23 phase IIb trial) (Sabchareon et al. 2012) nor in CYD15 (in which all subjects were aged ≥9 years). The pooled relative risks of hospitalization for dengue during the first year of long-term follow-up across CYD14, CYD15, and CYD57 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, those aged <9, and those aged ≥9 years, respectively (for details, see Hadinegoro et al. 2015).

Three interconnected hypotheses were proposed to explain the year 3 observations in the children aged <9 years in the CYD14 trial (Guy and Jackson 2016). Principally, temporal clustered vaccination, especially in seronegative participants, may result in a lower-level cross-reactive immune response prone to waning in a condensed period of time. If, as proposed above, vaccination in seronegative participants partially represents an attenuated subclinical primary exposure, this would be temporally clustered in younger vaccine recipients (aged <9 years) who are more likely to be seronegative. Contrastingly, among younger controls, primary wild-type infection and subsequent secondary exposure to a heterologous serotype (potentially, with a more severe outcome) would be temporally distributed. This "accelerated second infection" or temporality among vaccine recipients is the basis of the "cluster hypothesis," which also considers a potentially age-independent impact on vaccine-induced qualitative responses and subsequent efficacy (Guy and Jackson 2016). Consequently, the imbalance observed in vaccine recipients aged <9 years, resulting in a higher relative risk of hospitalization for dengue during year 3 in this age group, would be temporary. Year 4 data from CYD14 support this hypothesis: The relative risk in children aged <9 years was lower in year 4 (1.19; 95% CI, 0.65–2.28) than in year 3 (1.58; 95% CI, 0.61–4.83) (Gailhardou et al. 2016; Hadinegoro et al. 2016). This trend

was especially marked in children aged 2–5 years in whom the relative risk of hospitalization for dengue decreased from 7.45 (1.15, 313.80) in year 3 to 1.42 (95% CI, 0.58–3.99) in year 4. The cumulative relative risk over the full study (i.e., from dose 1 to year 4) was 0.79 (95% CI, 0.56–1.13) in children aged <9 years, showing a positive benefit for the vaccine, even in this younger population. Potential sensitization would no longer be present after a "second" infection caused by the booster effect of infection, and long-term benefits would be expected even in the initially seronegative population (Coudeville et al. 2016; Guy and Jackson 2016). However, there is a possibility that cases among young vaccine recipients may simply be because of declining immunity, which kinetics may differ between vaccine-induced and natural immunity, rather than an increased risk of severe disease. In children aged ≥9 years, significant

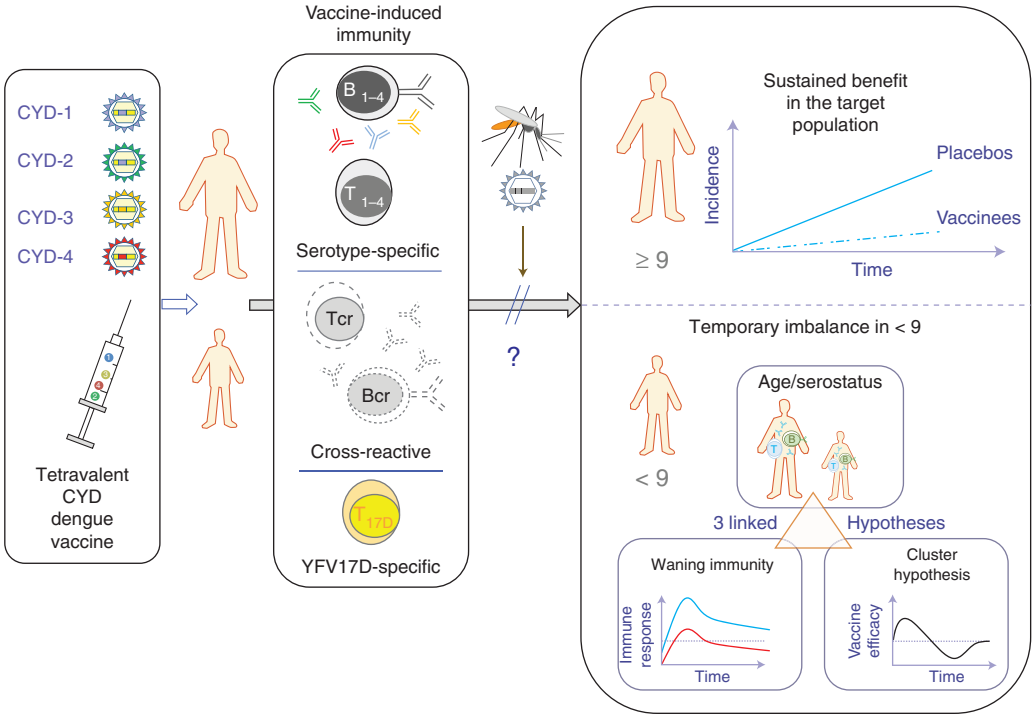

Figure 2. How concerned should we be about enhanced disease after vaccination? As described here, the nature/quality (e.g., specificity, profile) and level of the response induced by the tetravalent dengue vaccine are influenced by individual host factors such as age and serostatus at baseline, with the latter related to the former. The immune response to vaccination influences vaccine efficacy and long-term relative risk of hospitalization/severe disease. After CYD-TDV (chimeric yellow fever dengue-tetravalent dengue vaccine) vaccination, a sustained reduction in the relative risk of hospitalization was noted in children aged ≥9 years compared with placebo recipients during the third and fourth years after the first injection (Hadinegoro et al. 2015, 2016). In contrast, a 58% increase in relative risk of hospitalization was noted in children aged <9 years during the third year after the first injection in the CYD14 Asian efficacy trial, with the increased risk highest in children aged 2–5 years (Hadinegoro et al. 2015). However, this relative risk decreased during the fourth year of follow-up (Hadinegoro et al. 2016), in agreement with three interconnected hypotheses raised to explain the increased risk observed during the third year in children aged <9 years, and which all proposed would be temporary. As explained in the text, these hypotheses are linked to age/serostatus (higher/better responses in older and primed individuals), more rapid waning efficacy in young seronegative children, and clustered vaccination (primary infection-like) in that latter population (Guy and Jackson 2016). CYD-1 to 4, recombinant yellow fever-17D/dengue vaccine virus serotype 1 to 4.

reduction in hospitalized and severe VCD persisted throughout with a cumulative relative risk of 0.39 (95% CI, 0.24–0.60) (Hadinegoro et al. 2016). These results and hypotheses are shown in Figure 2.

Importantly, the clinical profile and associated viremia of severe hospitalized symptomatic VCD cases during the long-term follow-up is not different to that observed during the active surveillance phase (Hadinegoro et al. 2015). Further investigations to assess the immunological profile of hospitalized/severe dengue cases (i.e., cytokine profile) have shown no differences irrespective of phase and age group (Guy et al. 2015b; Harenberg et al. 2016), with no particular or enhanced disease profile linked to vaccination.

No relationship was observed in vitro between serotype-specific antibody-dependent enhancement (ADE) activity in vaccine recipients' sera and the level of efficacy against the corresponding serotype (Byers et al. 2015). This challenges the argument that ADE could be linked to lower or zero protection against symptomatic disease, and by extension that higher ADE activity in vaccine versus placebo recipients could explain the increase in hospitalized cases in year 3 among children aged <9 years who received the vaccine. This question remains a postulation at this stage.

Overall, sensitization to second/subsequent dengue infection resulting in more severe disease can be determined using the ratio of hospitalized cases to total VCD cases, as discussed above. If vaccination of seronegative individuals is compared with subclinical primary exposure, then an assessment of vaccine-induced sensitization requires a comparison between vaccine recipients who were seronegative before vaccination and those who then acquire a primary VCD, and controls who displayed a monotypic PRNT response at enrollment and then acquired a second VCD episode. This assessment requires the actual number of VCD cases and not predictions based on past experience. The long-term follow-up in the phase III efficacy trials captures hospitalized cases only, precluding such an assessment. Additional investigations will be conducted on the basis of an expanded surveillance period including both hospitalized and nonhospitalized cases; in fact, protocols have now been amended to return the trials to active surveillance. Additional data are also needed to further address the impact of serostatus on long-term efficacy and safety.

CONCLUDING REMARKS

Dengue is a complex disease, and both short- and long-term safety and efficacy of the vaccine has to be considered further to determine the overall benefit for human health. These will be addressed by ongoing long-term follow-up and future postlicensure studies. Initial introduction of CYD-TDV will be accompanied by long-term phase IV studies planned in collaboration with national authorities, which will serve to reinforce the medical value, impact, and feasibility of vaccination. In particular, a pharmacovigilance risk management plan (RMP) has been established in this regard. In conclusion, the development and implementation of a dengue vaccine requires long-standing and continuous efforts from private and public organizations to eventually bring a solution to the still-growing problem represented by dengue.

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