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WHO Report

Clinical development and regulatory points for consideration for second-generation live attenuated dengue vaccines

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ABSTRACT

Licensing and decisions on public health use of a vaccine rely on a robust clinical development program that permits a risk-benefit assessment of the product in the target population. Studies undertaken early in clinical development, as well as well-designed pivotal trials, allow for this robust characterization. In 2012, WHO published guidelines on the quality, safety and efficacy of live attenuated dengue tetravalent vaccines. Subsequently, efficacy and longer-term follow-up data have become available from two Phase 3 trials of a dengue vaccine, conducted in parallel, and the vaccine was licensed in December 2015. The findings and interpretation of the results from these trials released both before and after licensure have highlighted key complexities for tetravalent dengue vaccines, including concerns vaccination could increase the incidence of dengue disease in certain subpopulations. This report summarizes clinical and regulatory points for consideration that may guide vaccine developers on some aspects of trial design and facilitate regulatory review to enable broader public health recommendations for second-generation dengue vaccines.

1. Introduction

The first dengue vaccine (CYD-TDV or Dengvaxia®, by Sanofi Pasteur) was licensed in December 2015, after decades of research and clinical development. Despite a significant global demand, dengue vaccine development has been difficult for several reasons, including the need for a tetravalent vaccine with efficacy against each of the four dengue virus (DENV) serotypes, the lack of

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representative animal models, and concerns about vaccine-induced immune enhancement as seen in natural infection [1,2]. While the successful registration of the first dengue vaccine represented a major milestone, there have also been setbacks. First, the results of the multi-center pivotal Phase 3 trials highlighted important limitations [3,4]. In these trials, in which three vaccine doses were given separated by six months, efficacy varied according to serotype, age and baseline dengue serostatus. Because a safety signal was observed in young children in one of the trials during longer-term follow-up, children below 9 years of age were subsequently excluded from the age-indication of this vaccine. In April 2016, the World Health Organization (WHO) recommended countries consider introducing the first licensed dengue vaccine only in settings with a high burden of dengue disease, for the age group of 9–45 years, with seroprevalence criteria in the target age group for vaccination of ideally >70% [5]. WHO issued this recommendation due to limited evidence supporting the efficacy, safety, and long-term performance of the vaccine in DENV-seronegative individuals age >9 years, concerns about an excess risk of hospitalized dengue in younger (2–5 years old) subpopulations, and lower efficacy in DENV-seronegative subpopulations included in the current license [6]. WHO and advisors called on the company to further interrogate the clinical trial data and conduct additional targeted studies to further analyze the issue of safety and risk for increased incidence of symptomatic infection among vaccinated seronegative persons, to be done as soon as possible [5–7]. A proposal for the necessary post-licensure studies to address the question of safety in seronegatives, including study designs, has been published [8].

On November 29, 2017, Sanofi Pasteur announced that it had used a new NS1 assay on sera taken after the 3rd dose and imputation methods in order to classify participants retrospectively into those likely to have been seronegative or seropositive at the time of the first vaccine dose. These results were used to estimate the long-term safety and efficacy of the vaccine by serostatus prior to vaccination [9]. The company found an increased risk of severe and hospitalized dengue associated with vaccination among seronegatives. The company has stated its intention to change the label so that individuals who have not been previously infected by dengue virus should not be vaccinated. The WHO Global Advisory Committee on Vaccine Safety and the WHO Secretariat published interim statements on December 7, 2017 [10], and December 22, 2017 [11], respectively. A full evidence review is now underway to revise the WHO position.

In 2012, WHO issued guidelines on the regulation of dengue vaccines, including their clinical development [12]. In light of the experience of clinical development and of trying to formulate evidence-based policy-making for the first licensed dengue vaccine, WHO convened a group of independent experts on March 21, 2017, to develop points for consideration for the clinical evaluation of second-generation dengue vaccines. Here we summarize the discussions and recommendations from this ad hoc consultation, which took place before the Sanofi Pasteur announcement, but the points for consideration are all the more relevant in light of the new information. These reflections, summarized in Box 1 may help vaccine developers, regulators and public health decision-makers in planning studies or evaluating data on dengue vaccines. It does not replace original WHO guidance [12], but provides additional perspectives.

Box 1 Points for consideration for the development of second-generation live attenuated dengue vaccines.

- Early clinical studies are valuable to evaluate the potential for interference between individual vaccine viruses and the impact on the development of type-specific versus heterotypic immunity.
- Measuring antibody neutralization activity remains the best method of defining dengue vaccine immunogenicity; however, current assays do not easily distinguish between type-specific antibodies, transient heterotypic antibody, and long-lasting heterotypic antibody. Given this uncertainty, the critical time point for assessment of immunogenicity as a correlate of durable protection should be more than 12 months after the last vaccine dose. Various research assays may be complementary.
- Controlled Human Infection Model (CHIM) trials can provide initial proof-of-concept that a vaccine may have potential for clinical benefit, but greater confidence is required in Dengue CHIM performance and challenge should be complete 12 months or more after the last vaccine dose.
- For licensure, in the absence of an accepted correlate of protection or risk, vaccine efficacy will need to be demonstrated based on clinical outcomes collected over a multi-year period (multiple dengue seasons) that support durable benefit.
- Pre-vaccination and post-vaccination blood samples should be collected and sera stored from all trial participants.
- Dengue serostatus at baseline is a critical variable, and safety and efficacy by serostatus should be presented in a stratified analysis.
- Active surveillance used to assess efficacy against all dengue disease and severe dengue disease should be in place preferably for at least 3–5 years after the last vaccine dose.
- Immunogenicity and efficacy results should be interpreted in the context of potential transient heterotypic immunity that could wane over time.

2. Findings from the trials of the first-generation dengue vaccine

The first licensed dengue vaccine, CYD-TDV or Dengvaxia®, is a recombinant live attenuated, tetravalent dengue vaccine based on the yellow fever 17D vaccine backbone. The structural genes (prM-E) of the YF17D virus vector are replaced by the structural genes of each the four DENV serotypes. The initial license was typically with an indication for individuals aged 9–45 years living in endemic areas. Licensure was based on two large multicenter Phase 3 trials conducted in Asia and Latin America with over 30,000 trial participants; serostatus at baseline was assessed in a subset of about 2000 subjects in each trial [3,4,13]. A post hoc analysis stratifying vaccine efficacy and safety by <9 and ≥9 years of age across all trials led to the age indication starting at 9 years of age even though two efficacy trials enrolled down to 2 or 4 years of age. CYD-TDV is now registered in 19 countries [14].

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There were several unexpected findings in the trials that challenged previously held ideas about how this vaccine protects and the groups in whom protection might be greatest, and raised concerns of late onset vaccine-associated enhanced disease, which were later corroborated by the company's new analysis.

(1) Balanced immunogenicity between dengue serotypes, defined as a geometric mean neutralizing antibody titer (GMT) as measured by standard plaque reduction neutralization test (PRNT), did not correlate with serotype-specific efficacy. In the Phase 3 trial in Asia, GMT measured 28 days after the third vaccine dose were 166, 355, 207, and 151 against DENV1, DENV2, DENV3, and DENV4, respectively; however, the respective vaccine efficacies were 54.5%, 34.7%, 65.2%, and 72.4% [3]. The highest GMT was raised against DENV2, while for this serotype the vaccine efficacy was the lowest; the reverse relationship was seen for DENV4. Similar trends were seen in the Phase 3 trial in Latin America [4]. A formal analysis of the relationship between the level of neutralizing antibody at day 28 after the final vaccine dose and subsequent risk of disease in the 13–25 months after the first dose has been published. While higher neutralizing antibodies were shown to be associated with a reduced risk of disease, there was no threshold by which protection by CYD-TDV could be reliably predicted [15].
(2) Vaccine efficacy varied significantly by prior DENV infection status [3,4]. In a post hoc, pooled analysis of the two Phase 3 trials, vaccine efficacy was 78.2% (95%CI 65.4%, 86.3%) in trial participants who were DENV-seropositive at baseline (hereafter referred to as "seropositive"), and 38.1% (95%CI -3.4%, 62.9%) in trial participants who were DENV-seronegative at baseline (hereafter referred to "seronegative"). In participants 9–16 years of age, vaccine efficacy in seronegatives was 52.5% (95%CI 5.9%, 76.1%).
(3) In the third year of follow-up (12–24 months after the last vaccine dose), participants aged 2–5 years in the Phase 3 trial in Asia had an increased risk of hospitalized dengue, compared to the placebo group, with a relative risk (RR) 7.5 (95%CI 1.2, 313.8) [7,13]. Overall, the RR of hospitalized dengue during the study based on data available as of October 2015, was 1.3 in the 2–5 year-old age group (95%CI 0.8, 2.1). The reason for this elevated risk in the 2–5 year age-group was incompletely understood at the time of licensure. One hypothesis proposed was that in seronegative individuals, the vaccine acts like an asymptomatic primary infection, priming vaccinees to experience a "secondary-like" clinical presentation upon a first natural exposure to DENV [16–18]. While a DENV infection with a given serotype is thought to provide lifelong protection against a second infection with that serotype, second wildtype DENV infections of a different serotype than the first are associated with more severe clinical outcomes [19]. Serostatus was correlated with age in the Phase 3 trials: in Asia, the proportion of participants who were seropositive at the time of first vaccination was 51% for 2–5 year-olds, 72% among 6–11 year-olds and 81% among 12–16 year-olds [3]. The findings of the additional analyses announced in late November 2017 revealed that the increased relative risk for more severe disease was independent of age. The analysis confirmed that CYD-TDV provided persistent protective benefit against severe dengue among seropositives, but for seronegatives, the analysis found that in the longer term there was about two times higher risk of more severe dengue and hospitalizations in vaccinated participants [9,10].
(4) Follow-up analyses in a limited number of seronegative vaccinees suggest type-specific neutralizing responses were limited mainly to DENV4 [20]. This finding suggests that the protection of seronegative subjects observed against DENV1, DENV2, and DENV3 may have resulted predominantly from heterotypic cross-protection, rather than type-specific monotypic protection. If the heterotypic cross-protection induced by vaccination is temporary, it may support the biologic mechanism for the increased risk seen initially in the 2–5 year-olds, the age group that had the highest proportion of seronegatives, and subsequently in seronegative participants of any age.
(5) It was also expected that the highest protection would be seen after subjects had received the full 3 doses of vaccine ("per protocol" analyses), but vaccine efficacy was found to be similar for "intention to treat" analyses that included all cases occurring after the first dose [3,4]. Whether this has implications for the ability of this or other live vaccines to boost the immune response at a later stage is currently unknown.

The first dengue vaccine has significant limitations in relation to large-scale public health use. An ideal dengue vaccine would offer high protection against clinical disease due to any serotype and would be similarly efficacious and safe regardless of prior DENV infection and age at vaccination. Protection would ideally be long-lasting, and vaccine-boosting of immunity should be possible if protection wanes over time. Additional dengue vaccine candidates are currently in advanced clinical development, and regulators and policy makers will want to ascertain whether or not second-generation candidates share similarities to CYD-TDV. Below we outline some considerations that may help with trial design and interpretation of clinical trial results, as well as in licensing and use decisions. Several of the considerations have already been integrated into ongoing dengue clinical development programs.

3. Learning from natural DENV infection

Tetravalent vaccination, representing simultaneous exposure to the four DENV antigens, is fundamentally different from sequential exposure to individual DENV serotypes through natural infection. Nevertheless, it is important to consider aspects of live viral vaccines through the lens of what is known about natural infection. Firstly, the DENV serotypes are four genetically and serologically distinct viruses [21]. A tetravalent vaccine, therefore, combines four live vaccines, with risk of interference between the components. Many years have been spent in early phases of live dengue vaccine development attempting to find the right formulation to overcome such interference [22].

Humoral immunity induced by infection with one DENV has been characterized as homotypic against the infecting serotype and heterotypic against the non-infecting other (and not previously seen) serotypes. While homotypic immunity is long-lasting, heterotypic immunity that protects against non-infecting serotypes is transient. It is believed that heterotypic immunity induced after a primary wildtype DENV infection lasts about 1–2 years, after which the individual is predisposed to more severe outcomes associated with a second wildtype infection with another serotype [23–25]. However, as 3rd and 4th DENV infections with different serotypes are rarely associated with severe disease, and it is assumed that heterotypic immunity following a second infection is long-lasting [26]. Thus, it is important to distinguish between transient and long-lasting heterotypic (also termed "multitypic" [27]) immunity, the latter likely based on broadly cross-neutralizing antibodies elicited by post-primary infections. Ideally, a dengue vaccine should elicit long-lasting type-specific antibodies against each of the four serotypes and/or long-lasting cross-neutralizing heterotypic antibodies.

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Preliminary data from cohort studies suggest the neutralization titers necessary for protection are higher than 1:10 [28,29], which has been accepted as a correlate of vaccine-induced protection for other flavivirus vaccines [30]. Thus, immunogenicity requirements for a dengue vaccine may be higher than have been estimated for other monovalent flavivirus vaccines, with the caveat that variations in laboratory assays inhibit a quantitative comparison between titers against different flaviviruses. A further complication is that thresholds of protection may vary by DENV serotype and by candidate vaccine formulation. This poses a significant challenge to assessing likely efficacy of second-generation dengue vaccines based only on immunogenicity, as well as the use of immunogenicity to bridge efficacy to populations not evaluated in clinical efficacy trials.

4. Considerations for early-stage clinical development of live attenuated dengue vaccines

Early phase clinical studies can help characterize potential interference between vaccine viruses, which influences whether vaccine-induced immunity is serotype-specific or heterotypic for each serotype. Additional characterization of vaccine candidates using different assays and approaches can be complementary and aid in de-risking late-stage development, helping to anticipate vaccine efficacy, longer-term protection, and safety in the field. None of the studies outlined below need be considered on the critical path to licensure but may bring added value in regulatory and policy-making assessments.

4.1. Neutralization assays

The limitations of existing neutralization assays have been described [29], but there are currently no validated alternatives. Neutralization assays using sera collected shortly after vaccination cannot distinguish between monotypic (long-lasting, type-specific), transient heterotypic, or long-lasting heterotypic (multitypic) antibodies [27]. In addition, they may not measure antibodies that may enhance infection. Therefore, any classical measurement of neutralizing antibodies must be interpreted in the context of time since exposure to infection or vaccination. While repeated measurements over time may offer better characterization of a post-vaccination immune response, the level(s) of antibody required for robust protection is not yet known. Data from any trial in which both pre-infection serological responses and disease outcomes have been measured can be used to help determine which assays should be further investigated for determining a correlate of protection.

There may be value in trying to optimize neutralization assays to best reflect the immune response elicited by vaccination, such as using Fcy-receptor bearing cells and measuring neutralization using a variety of viral strains, including low-passage strains from more recent clinical isolates. However, these modifications are still considered exploratory. It is noted that non-neutralizing antibodies may also contribute to protection; for instance, dengue NS1 interacts with the complement system and may directly contribute to the vascular permeability syndrome, while antibodies to NS1 can confer protection by blocking its pathogenic effects [27,31].

4.2. Vaccine infectivity and interference

Live attenuated vaccines elicit human immune responses through vaccine viral replication. Thus, for a tetravalent vaccine to generate type-specific immune responses to each of the four serotypes, it is thought that each of the four vaccine components should replicate within the host. Demonstrating replication for each of the four vaccine viruses, even within the same individual, when administered as a live tetravalent vaccine, is highly desirable given the potential risk of interference.

The ability of vaccine viruses to replicate and the quality of vaccine-induced immunity can be measured by multiple means, and such studies are important to perform, at least initially, in a flavivirus-naïve study population. Potential options include: (1) recovering and quantifying vaccine virus through culture (e.g. [32]); (2) detecting and quantifying viral RNA by RT-PCR (e.g. [33]), ideally by detecting negative strand RNA since this indicates viral replication, not just presence of vaccine virus; and (3) antibody depletion assays to determine whether type-specific responses were generated for each of the four serotypes (e.g. [20]). It is acknowledged that there are challenges with assay sensitivity, and recovering and quantifying vaccine virus from each of the four serotypes in an individual is a high bar. At a minimum, indicating the proportion of a vaccinated group with detectable replication of each vaccine virus provides basic characterization, particularly when this is conducted serially over 14 days after the first vaccine dose (e.g. [34]). The more evidence generated to support type-specific immune responses against the multiple vaccine viruses, the more confidence regulators and policy makers may have in their assessment of long-term efficacy and public health utility.

Another approach has been to study immunogenicity of each monovalent vaccine separately, as well as in combination, and to compare levels of serotype-specific antibody. A problem with this approach is the limitations of neutralization tests to measure type-specific long-lasting protective antibody, especially at time points close to vaccination. Furthermore, viral replication kinetics of monovalent candidates may differ when formulated as a tetravalent vaccine and it would be important to conduct studies early in development defining these kinetics over a broad range of days following vaccination. Longer-lasting antibodies, beyond one year, may be more indicative of protection. Additionally, studies in non-human primates, especially challenge studies demonstrating protection against viraemia at a meaningful time-point (e.g., >12 months after the last vaccine dose), are of value, noting limitations in extrapolating from the animal model to humans.

4.3. Controlled human infection model

Although not required as part of the critical path for vaccine licensure, controlled human infection model (CHIM) trials can provide initial proof-of-concept that a vaccine is likely to have clinical benefit, and this may de-risk decisions to evaluate candidates in large Phase 3 efficacy trials. There is also the potential for dengue CHIM to assist in the identification of an immune correlate of risk or protection and potentially expand the indication for a vaccine. There are currently both attenuated infection models (which do not cause disease) and disease models [35]. In all models, the virus is administered parenterally, which potentially confounds the interpretation of outcomes, as the induction of infection via mosquito bite extends the incubation period and allows both innate and adaptive immunity to condition the course of the infection. The validity of efficacy measured by CHIM has yet to be established, but an initial assessment can be made once efficacy results from a Phase 3 trial are available for one vaccine candidate that has also been evaluated by CHIM [36,37]. How well CHIM efficacy estimates will predict efficacy against wild-type virus may also differ between the infection and disease model.

Challenge strains have not yet been developed for all DENV serotypes, which would be needed for a comprehensive assessment. CHIM studies with each of the four serotypes would incur high costs, and DENV serotypes for which there may be *a priori* concern about interference, such as those with lower seroconversion rates,

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could be prioritized. Additionally, the timing of challenge is critical in the interpretation of results due to the potential for short-lived cross-protection. The longest time interval for a dengue CHIM between vaccination and challenge is currently 3.5 years [38]. Ideally, volunteers would be challenged at 12 months or greater after vaccination, after transient heterotypic immunity would be expected to have waned. Otherwise, it is possible that even high efficacy demonstrated through CHIM could reflect the presence of transient heterotypic protection.

5. Considerations for late-stage clinical development

Until a surrogate or correlate of protection is established [30], pivotal efficacy trials of dengue vaccines will need to be conducted based on a clinical endpoint. The licensure of the first dengue vaccine introduces additional complexities to the design and site selection for second-generation vaccine development and will require close consultation with national regulatory authorities.

Efficacy trials of dengue vaccines have specified a primary endpoint of virologically-confirmed dengue of any severity due to any serotype. Even with these broad endpoints, it has been necessary to include 10,000–20,000 participants in trials to have statistical power to demonstrate vaccine efficacy. Such trials have very high cost and it is important that the designs of Phase 3 pivotal trials are optimized based on lessons learned from the first pivotal trials.

The objectives of a clinical development program for a dengue vaccine are likely to remain largely as outlined in the initial WHO guidelines [12], with the addition of critical analyses of immunological and clinical outcomes by pre-vaccination immune status. Thus, the clinical evaluation of a candidate live tetravalent dengue vaccine should document (1) the immune responses elicited by the vaccine against each of the four DENV serotypes according to pre-vaccination immune status; (2) vaccine efficacy for the prevention of symptomatic dengue of any severity caused by any DENV serotype over an appropriate minimum period of observation (preferentially 5 years) according to pre-vaccination dengue immune status; and (3) the safety profile according to pre-vaccination immune status. As further outlined in the WHO guideline, preliminary evidence should be gathered showing the absence of a significant signal that the immediate and longer-term immune response to a candidate dengue vaccine predisposes vaccinated individuals (or a subset) to an increased risk of dengue and/or severe dengue disease with subsequent natural infections, relative to the control group. Finally, surrogate markers or immune correlates of protection and/or risk should be defined [12].

5.1. Immune status at vaccination

It is essential that a pivotal trial be designed so that vaccine efficacy, safety, and duration of protection can be assessed by serotype and serostatus. The finding of a substantially increased risk of hospitalized dengue associated with vaccination of seronegatives in the longer-term following the last dose of the CYD-TDV vaccine [11] has led to increased focus on vaccine efficacy and safety in seronegative populations. Biologically this is not surprising, as serostatus is a strong modifier of immune responses, infection and disease susceptibility, and vaccine take. It is now clear that vaccine efficacy and clinical outcomes should always be evaluated stratified by baseline neutralizing antibody to DENV. In some cases, it may also be appropriate to consider the influence of prior infection with other prevalent flaviviruses [39,40].

It has been proposed, and it is generally accepted, that in future Phase 3 trials of dengue vaccines, pre-vaccination and post-vaccination blood samples should be collected and sera stored from all trial participants, not just a subset [29]. Should testing the entire population at the onset be financially and logistically unfeasible, testing a large subset or testing the prior stored samples for all cases would provide information on efficacy and safety according to prior serostatus. To help characterize the immune response generated by vaccination over time, blood samples should be collected at different time points after vaccination, e.g. between doses, after the last dose, 6 months after the last dose, 12 months after the last dose, and at 12-month intervals for the duration of the trial and follow-up. Additionally, given that the role of T cell immunity in vaccine-induced immunity remains unclear, it is desirable to also collect PMBCs from as large a subset of participants as possible for exploratory analyses.

In order to have sufficient seronegative trial participants to characterize the risk/benefit in this subpopulation, the study population could be enriched for seronegative participants, or a trial could be powered to demonstrate efficacy in seronegatives. In the two Phase 3 trials of CYD-TDV, the incidence of virologically confirmed dengue was similar in unvaccinated seronegatives and seropositives (in the Asia trial, incidences of 4.3% (95%CI 2.5–6.6) and 3.9% (95%CI 2.7–5.4) in seronegatives and seropositives, respectively [3], and in the Latin American trial corresponding incidences of 3.2% (95% 1.5–5.9) and 2.3% (1.5–3.5) [4]). The seropositive population may have included some individuals who had already experienced 2 or more infections at the time of vaccination, a group in which we would expect little additional public health value for a vaccine given the low rate of disease associated with tertiary and quaternary infections. In the same epidemiological context as the Asian trial, 70% efficacy could be demonstrated in seronegatives with as few as ~1200 trial participants (1-sided alpha = 2.5% and 90% power, lower limit of the 95%CI of 0). Seronegative study participants could be identified by screening using ELISA, which is less costly and labor-intensive than PRNT. While available ELISA kits are not specific enough to determine baseline seropositivity given the potential cross-reactivity with other flaviviruses, they could be adequate to rule out prior exposure to any flavivirus, including DENV.

5.2. Methods and duration of follow-up

If a live attenuated vaccine stimulates immunity similarly to natural infection, replication of only one component in a tetravalent vaccine may demonstrate efficacy against all serotypes for the first 1–2 years after the last dose of vaccine [23–25]. Thus, vaccine efficacy estimates against each of the four serotypes generated in a 12-month time frame after the last dose should be interpreted in the context of potentially transient cross-protection. Twelve months post-vaccination is a typical primary endpoint for pivotal trials for other vaccines, but for DENV, a longer period of follow-up is needed to establish both safety and the longer-term efficacy over multiple dengue seasons, going beyond temporary cross-protection. Hence, WHO guidelines recommend subjects to be followed-up for safety and efficacy for at least 3–5 years from the time of completion of primary vaccination [12]. Registration may be sought on the basis of early follow-up data (e.g. 2 years after last dose), but follow-up should continue with timely updates provided to regulators throughout their assessment. It is important that the same level of surveillance be maintained throughout the duration of the study so that changes in vaccine efficacy over time can be detected particularly as cross-protection wanes. To maintain the integrity of the trial over this extended timeframe, trial participants and investigators who interact with the participants should remain blinded unless there is an ethical obligation to inform participants, such as in the post-licensure phase when and if there is a national recommendation for vaccination. The method of case detection should ideally remain unchanged (i.e., active surveillance) to make meaningful comparisons over time. The primary

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endpoint for vaccine trials has typically been virologically-confirmed dengue of any severity as measured by active surveillance in order to have a manageable sample size, as hospitalized or severe dengue is relatively rare. Capturing both dengue of any severity as well as hospitalized and well-defined severe dengue allows for an understanding of whether dengue illness presentation is modified by vaccination status, which may vary by time since last vaccination. Clinical data should be carefully monitored for any imbalance of symptomatic or severe dengue cases, including in subpopulations, and in particular in the period following expected cross-protection.

An extended follow-up period will also allow for additional power to look at secondary analyses, such as sustained vaccine efficacy by infecting serotype(s). It is desirable that clinical trial sites are chosen such that there is circulation of each of the four serotypes within the trial, likely requiring a geographically dispersed multicenter study.

6. Other regulatory and policy considerations

Registration and public health recommendations would be facilitated by data that indicate presence of protective monotypic responses against all vaccine strains, as well as vaccine efficacy beyond the period of short-lived heterologous cross-protection, which is likely beyond 12 months after the last dose administered. Even if serotype-specific efficacy and efficacy in seronegatives are not primary endpoints (as is the case for the two candidates currently in Phase 3 trials [37,41]), regulators may require data that provide reassurance that the vaccine can be used safely and effectively in the target population. Any public health recommendations for use should also take into account such data.

For some flaviviruses (e.g., Japanese encephalitis), immunobridging has been accepted for licensing across vaccine candidates/platforms; however, these vaccines are monovalent rather than tetravalent. In the context of dengue vaccines, due to the lack of clear understanding of mechanisms of protection and risk, the variability in neutralization assays, the lack of assays that properly distinguish various types of neutralizing antibody, and the likely differences in elicited immunity by different dengue vaccine candidates, immunobridging based on traditional PRNT may be questionable to regulators as the primary basis of licensure. Assays able to measure serotype-specific long-lasting protective immunity are critically needed. Currently, placebo-controlled efficacy trials are still likely to be considered ethical and desirable in many settings. WHO has provided guidance on ethical acceptability of placebo-controlled trials in the context of the availability of a licensed product [42].

7. Conclusions

Our understanding of how dengue vaccines can decrease, or increase, the risk of dengue disease is evolving. To ensure that regulatory and public health-decision makers can best and rapidly utilize the vaccination tools that become available, a robust clinical development program is needed that affords adequate characterization of a vaccine candidate's benefit-risk profile over a period of time sufficient to predict durable benefit. There are models for joint regulatory assessments that may aid national regulatory authorities with complex dossiers, and this was done for the first licensed dengue vaccine [43]. Clinical data generated in the course of a clinical development program should be made publicly available for regulators, policy-makers, and the broader community in a timeframe consistent with WHO's position on clinical trial results reporting [44].

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Declarations

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of or reflecting the position of the agencies or organizations with which the authors are affiliated. JH is a staff member of the World Health Organization, as was KV at the time of this work. AWS is consultant to the WHO on matters related to arboviral diseases. BI is an employee of PATH; he is a former employee of GSK. EH served on the Scientific Advisory Board of Sanofi Pasteur during the Phase 3 vaccine trials, and her laboratory received research funds from Takeda Vaccines, Inc. to analyze samples from vaccine recipients. PGS is a member of the Independent Data Monitoring Committee for trials of the CYD-TDV vaccine. APD has consulted with Merck & Co on dengue vaccines. ST serves on advisory and safety boards for Sanofi Pasteur and Takeda Vaccines, Inc., and has performed consulting work for GSK Vaccines and Merck & Co. ADS has consulted on dengue vaccines for Takeda, Merck and Glaxo Smith Kline. His group has received funding from Sanofi and Takeda to analyze vaccine induced immune responses. He is listed as an inventor on patents related to flavivirus vaccines and diagnostics. WS's contributions are informal communications and represent his best judgment. These comments do not bind or obligate the U.S. FDA. MC is a full time employee of the EMA. KC is a staff member of the Brazilian Health Regulatory Agency – Anvisa. The views and opinions here expressed should not be used in place of regulations, published guidance documents or direct discussions with Anvisa.

References

[1] Thomas SJ. Developing a dengue vaccine: progress and future challenges. Ann N Y Acad Sci 2014;1323:140–59. https://doi.org/10.1111/nyas.12413.
[2] Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, et al. Antibody-dependent enhancement of severe dengue disease in humans. Science 2017. https://doi.org/10.1126/science.aan6836.
[3] Capeding MR, Tran NH, Hadinegoro SRS, Ismail HIHJM, 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 Lond Engl 2014;384:1358–65. https://doi.org/10.1016/S0140-6736(14)61060-6.
[4] Villar L, Dayan GH, Arredondo-García 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;372:113–23. https://doi.org/10.1056/NEJMoa1411037.
[5] World Health Organization. Dengue vaccine: WHO position paper – July 2016. Releve Epidemiol Hebd 2016;91:349–64.
[6] Wilder-Smith A, Vannice KS, Hombach J, Farrar J, Nolan T. Population Perspectives and world health organization recommendations for CYD-TDV dengue vaccine. J Infect Dis 2016;214:1796–9. https://doi.org/10.1093/infdis/jiw341.
[7] World Health Organization. Background Paper on Dengue Vaccines. <http://www.who.int/immunization/sage/meetings/2016/april/1_Background_Paper_Dengue_Vaccines_2016_03_17.pdf>; 2016.
[8] Wichmann O, Vannice K, Asturias EJ, de Albuquerque Luna EJ, Longini I, Lopez AL, et al. Live-attenuated tetravalent dengue vaccines: The needs and challenges of post-licensure evaluation of vaccine safety and effectiveness. Vaccine 2017;35:5535–42. https://doi.org/10.1016/j.vaccine.2017.08.066.
[9] Sanofi Pasteur. Sanofi updates information on dengue vaccine. <http://mediaroom.sanofi.com/sanofi-updates-information-on-dengue-vaccine/>; 2017 [accessed November 29, 2017].
[10] World Health Organization. GACVS Statement on Dengvaxia ® (CYD-TDV). <http://www.who.int/vaccine_safety/committee/GACVS-StatementonDengvaxia-CYD-TDV/en/>; 2017 [accessed January 12, 2018].
[11] World Health Organization. Updated Questions and Answers related to the dengue vaccine Dengvaxia® and its use. WHO 2017. <http://www.who.int/

immunization/diseases/dengue/q_and_a_dengue_vaccine_dengvaxia_use/en/>; 2017 [accessed December 22, 2017].
[12] World Health Organization. Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). <http://who.int/biologicals/areas/vaccines/TRS_979_Annex_2.pdf>; 2012.
[13] 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. https://doi.org/10.1056/NEJMoa1506223.
[14] Sanofi Pasteur. Dengue Vaccine Registered in 19 Countries <http://dengue.info/dengue-vaccine-registered-in-19-countries/> [accessed November 2, 2017].
[15] Moodie Z, Juraska M, Huang Y, Zhuang Y, Fong Y, Carpp LN, et al. Neutralizing antibody correlates analysis of tetravalent dengue vaccine efficacy trials in Asia and latin America. J Infect Dis 2018;217(5):742–53. https://doi.org/10.1093/infdis/jix609.
[16] Guy B, Jackson N. Dengue vaccine: hypotheses to understand CYD-TDV-induced protection. Nat Rev Microbiol 2016;14:45–54. https://doi.org/10.1038/nrmicro.2015.2.
[17] Ferguson NM, Rodríguez-Barraquer I, Dorigatti I, Mier-Y-Teran-Romero L, Laydon DJ, Cummings DAT. Benefits and risks of the Sanofi-Pasteur dengue vaccine: Modeling optimal deployment. Science 2016;353:1033–6. https://doi.org/10.1126/science.aaf9590.
[18] Flasche S, Jit M, Rodríguez-Barraquer I, Coudeville L, Recker M, Koelle K, et al. The long-term safety, public health impact, and cost-effectiveness of routine vaccination with a recombinant, live-attenuated dengue vaccine (Dengvaxia): A model comparison study. PLoS Med 2016;13:e1002181. https://doi.org/10.1371/journal.pmed.1002181.
[19] Endy TP, Yoon I-K, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. Curr Top Microbiol Immunol 2010;338:1–13. https://doi.org/10.1007/978-3-642-02215-9_1.
[20] Henein S, Swanstrom J, Byers AM, Moser JM, Shaik SF, Bonaparte M, et al. Dissecting antibodies induced by a chimeric yellow fever-dengue, live-attenuated, tetravalent dengue vaccine (CYD-TDV) in naive and dengue-exposed individuals. J Infect Dis 2017;215:351–8. https://doi.org/10.1093/infdis/jiw576.
[21] Guzman MG, Harris E. Dengue. Lancet 2015;385:453–65. https://doi.org/10.1016/S0140-6736(14)60572-9.
[22] Edelman R, Wasserman SS, Bodison SA, Putnak RJ, Eckels KH, Tang D, et al. Phase I trial of 16 formulations of a tetravalent live-attenuated dengue vaccine. Am J Trop Med Hyg 2003;69:48–60.
[23] 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:e2357. https://doi.org/10.1371/journal.pntd.0002357.
[24] Reich NG, Shrestha S, King AA, Rohani P, Lessler J, Kalayanarooj S, et al. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. J R Soc Interface 2013;10:20130414. https://doi.org/10.1098/rsif.2013.0414.
[25] Anderson KB, Gibbons RV, Cummings DAT, 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;209:360–8. https://doi.org/10.1093/infdis/jit436.
[26] Gibbons RV, Kalanarooj S, Jarman RG, Nisalak A, Vaughn DW, Endy TP, et al. Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. Am J Trop Med Hyg 2007;77:910–3.
[27] Halstead SB. Identifying protective dengue vaccines: guide to mastering an empirical process. Vaccine 2013;31:4501–7. https://doi.org/10.1016/j.vaccine.2013.06.079.
[28] 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:e3230. https://doi.org/10.1371/journal.pntd.0003230.
[29] Katzelnick LC, Harris E. Participants in the summit on dengue immune correlates of protection. Immune correlates of protection for dengue: State of the art and research agenda. Vaccine 2017;35:4659–69. https://doi.org/10.1016/j.vaccine.2017.07.045.
[30] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol CVI 2010;17:1055–65. https://doi.org/10.1128/CVI.00131-10.
[31] Beatty PR, Puerta-Guardo H, Killingbeck SS, Glasner DR, Hopkins K, Harris E. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci Transl Med 2015;7:304ra141. https://doi.org/10.1126/scitranslmed.aaa3787.
[32] Kirkpatrick BD, Durbin AP, Pierce KK, Carmolli MP, Tibery CM, Grier PL, et al. Robust and balanced immune responses to All 4 dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, flavivirus-naive adults. J Infect Dis 2015;212:702–10. https://doi.org/10.1093/infdis/jiv082.
[33] Mantel N, Aguirre M, Gulia S, Girerd-Chambaz Y, Colombani S, Moste C, et al. Standardized quantitative RT-PCR assays for quantitation of yellow fever and chimeric yellow fever-dengue vaccines. J Virol Methods 2008;151:40–6. https://doi.org/10.1016/j.jviromet.2008.03.026.
[34] Eckels KH, Thomas SJ, De La Barrera R, Carletti I, Fernandez S, Dessy F, et al. A phase II, randomized, safety and immunogenicity study of a re-derived, live-attenuated dengue virus vaccine in healthy adults. Am J Trop Med Hyg 2013;88:73–88. https://doi.org/10.4269/ajtmh.2012.12-0361.
[35] Larsen CP, Whitehead SS, Durbin AP. Dengue human infection models to advance dengue vaccine development. Vaccine 2015;33:7075–82. https://doi.org/10.1016/j.vaccine.2015.09.052.
[36] Kirkpatrick BD, Whitehead SS, Pierce KK, Tibery CM, Grier PL, Hynes NA, et al. The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. Sci Transl Med 2016;8:330ra36. https://doi.org/10.1126/scitranslmed.aaf1517.
[37] Phase III Trial to Evaluate Efficacy and Safety of a Tetravalent Dengue Vaccine. <https://clinicaltrials.gov/ct2/show/NCT02406729>[accessed November 2, 2017].
[38] Sun W, Eckels KH, Putnak JR, Lyons AG, Thomas SJ, Vaughn DW, et al. Experimental dengue virus challenge of human subjects previously vaccinated with live attenuated tetravalent dengue vaccines. J Infect Dis 2013;207:700–8. https://doi.org/10.1093/infdis/jis744.
[39] Scott RM, Eckels KH, Bancroft WH, Summers PL, McCown JM, Anderson JH, et al. Dengue 2 vaccine: dose response in volunteers in relation to yellow fever immune status. J Infect Dis 1983;148:1055–60.
[40] Anderson KB, Gibbons RV, Thomas SJ, Rothman AL, Nisalak A, Berkelman RL, et al. Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. PLoS Negl Trop Dis 2011;5:e1311. https://doi.org/10.1371/journal.pntd.0001311.
[41] Efficacy, Safety and Immunogenicity of Takeda's Tetravalent Dengue Vaccine (TDV) in Healthy Children. <https://clinicaltrials.gov/ct2/show/NCT02747927> [accessed November 2, 2017].
[42] Rid A, Saxena A, Baqui AH, Bhan A, Bines J, Bouesseau M-C, et al. Placebo use in vaccine trials: recommendations of a WHO expert panel. Vaccine 2014;32:4708–12. https://doi.org/10.1016/j.vaccine.2014.04.022.
[43] Vannice K, Chocarro L, Pfleiderer M, Bellah A, Ward M, Yoon I-K, et al. The value of multi-country joint regulatory reviews: The experience of a WHO joint technical consultation on the CYD-TDV (Dengvaxia®) dossier. Vaccine 2017;35:5731–3. https://doi.org/10.1016/j.vaccine.2017.07.044.
[44] Moorthy VS, Karam G, Vannice KS, Kieny M-P. Rationale for WHO's new position calling for prompt reporting and public disclosure of interventional clinical trial results. PLoS Med 2015;12:e1001819. https://doi.org/10.1371/journal.pmed.1001819.

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