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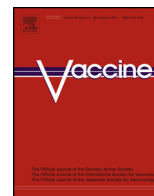
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## Long-term safety assessment of live attenuated tetravalent dengue vaccines: Deliberations from a WHO technical consultation <sup>☆</sup>

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### ABSTRACT

Dengue is a rapidly growing public health threat with approximately 2.5 billion people estimated to be at risk. Several vaccine candidates are at various stages of pre-clinical and clinical development. Thus far, live dengue vaccine candidates have been administered to several thousands of volunteers and were well-tolerated, with minimal short-term safety effects reported in Phase I and Phase II clinical trials. Based on the natural history of dengue, a theoretical possibility of an increased risk of severe dengue as a consequence of vaccination has been hypothesized but not yet observed. In October 2011, the World Health Organization (WHO) convened a consultation of experts in dengue, vaccine regulation and vaccine safety to review the current scientific evidence regarding safety concerns associated with live attenuated dengue vaccines and, in particular, to consider methodological approaches for their long-term evaluation. In this paper we summarize the scientific background and methodological considerations relevant to the safety assessment of these vaccines. Careful planning and a coordinated approach to safety assessment are recommended to ensure adequate long-term evaluation of dengue vaccines that will support their introduction and continued use.

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### 1. Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease globally, with more than a 30-fold increase in the annual number of cases reported to WHO in the last 50 years and increasing numbers of countries affected, including both urban and rural settings [1,2]. Dengue virus, belonging to the Flavivirus genus, has four antigenically distinct serotypes (DENV1–4), each of which is

capable of causing disease in humans when transmitted through the bite of *Aedes* mosquitoes. The clinical disease usually manifests as an acute febrile illness, or dengue fever, but occasionally develops into severe dengue. WHO estimates that approximately 2.5 billion people are at risk from dengue, and 50–100 million dengue infections occur annually of which about 500,000 are cases of severe dengue requiring hospitalization [3]. There is no licensed dengue vaccine and prevention is exclusively through vector control. Successful introduction of safe and effective dengue vaccines will be a critical step in preventing dengue infection and disease. Several vaccine candidates are in pre-clinical development, and some are undergoing clinical trials [4–6]. The most advanced live attenuated tetravalent dengue vaccine (LATDV) candidate, which is currently undergoing Phase III clinical trials, is a chimeric vaccine combining the preM and E genes of dengue with the yellow fever 17D-backbone [7].

While dengue pathogenesis is recognized to be complex, several viral, clinical and epidemiological studies have advanced the understanding of aspects critical to vaccination as summarized in previous reviews [8–11]. Infection with a specific DENV serotype

*Abbreviations:* ADE, antibody-dependent enhancement; DENV, dengue virus; LATDV, live attenuated tetravalent dengue vaccine; PBMC, peripheral blood mononuclear cells; SAE, serious adverse event; WHO, World Health Organization.

<sup>☆</sup> *Disclaimer:* This report contains the collective views of an international group of experts. The authors alone are responsible for the views expressed and they do not necessarily represent the decisions, policy or views of the World Health Organization.

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produces immunity to that serotype that is thought to be life-long but only short-term protection, of 3–6 months, against other serotypes. The most important risk factor for severe disease is previous infection, most commonly after a second (and rarely third) infection with a different DENV serotype than the previous infection(s) [12,13]. Severe disease is 15–80 times more common in secondary compared to primary infections [14]. Observations in dengue-infected infants born to dengue-immune mothers suggest an increased risk for severe disease associated with decline of maternally derived neutralizing antibodies to sub-neutralizing concentrations [15]. These observations and other epidemiological evidence underlie the concept of antibody-dependent enhancement (ADE) of disease as a primary mechanism triggering the development of severe disease in secondary infections [9,15,16].

Different mechanisms for the immune enhancement of disease are hypothesized to contribute to severe dengue. “Extrinsic ADE” is believed to result from non-neutralizing antibodies or neutralizing antibodies at concentrations below their neutralization capacity that enhance viral entry into host cells by forming complexes with the virus and binding to Fc-receptors on the host cells [16]. In addition, attachment and entry of virus-antibody complexes into Fc receptor-bearing cells has been proposed to modify innate and adaptive intracellular antiviral mechanisms and enhance replication (“intrinsic ADE”) [15]. Other mechanisms of immune enhancement involving cellular immune responses have been suggested [17,18]. The risk of severe disease is thought to depend on the balance and nature of the T-cell response which may favour anti-viral activity or the secretion of pro-inflammatory cytokines that facilitate plasma leakage. These hypothesized mechanisms of immune enhancement are not unrelated because antibody-mediated increase of viral replication would result in a greater number of dengue-infected cells that would elicit a greater T-cell response, and presumably a stronger but aberrant immune response leading to more severe disease. In addition, large amounts of NS1 generated by enhanced DENV replication could bind to serum complement with generation of anaphylatoxin C5a and increased vascular leakage [19].

A key challenge in the development of dengue vaccines is the limited understanding of the complexity of dengue immunology and pathogenesis, including a potential risk of sensitization to severe disease (i.e., immune enhancement) following vaccination. Ongoing clinical trials for candidate LATDVs, taking into account the relevant WHO guidance for long term assessment of dengue vaccines [4,20], will assess the potential risk of immune enhancement for periods of up to 5 years, however more robust information will be needed from post-licensure studies.

Post-licensure vaccine introduction strategies will need to consider assessments of the long-term safety and effectiveness of the vaccine in addition to other common parameters for vaccination such as the target population (age groups and regions), vaccine delivery (e.g., mass vaccination campaigns or routine immunization with or without catch-up campaigns), scheduling, use in special populations not included in clinical trials, coverage targets, and co-administration with other vaccines.

With the first LATDV candidate now in Phase III trials there is a need for further, more detailed, guidance on strategies for long-term monitoring of vaccine safety. WHO convened an expert consultation in October 2011 to initiate review of the current data regarding potential safety risks associated with LATDVs. Experts reviewed aspects of the post-licensure evaluation of LATDVs, including potential diagnostic approaches for dengue and study design issues. This paper summarizes the key deliberations and conclusions of the consultation. Candidate dengue vaccines based on other technologies in earlier stages of clinical development or in preclinical studies [6] were not discussed but many of the considerations discussed here may also apply.

## 2. Dengue vaccine-specific safety considerations

Current WHO guidelines recommend that safety assessment of dengue vaccines should include follow-up of dengue-vaccinated and control subjects for at least 3–5 years after completion of primary vaccination in Phase II and Phase III trials [4,20]. Evidence from settings with different mosquito transmission intensities will be desirable as boosting from natural infection and different circulating serotypes and genotypes may impact on vaccine safety.

Present LATDV candidates have been shown to induce very low levels of viraemia and have been well-tolerated in healthy flavivirus-naïve adults [21–23]. In children, some studies showed more local and systemic reactions after the first dose than after subsequent doses and fever was more frequent than in adults [23–26]. However, no dengue-like illness caused by the vaccine has been reported in clinical trials of current candidate vaccines. Nonetheless, the risk of dengue-like illness following vaccination remains a potential safety concern for LATDVs, and the safety profile will need to be corroborated in special populations, such as HIV-infected and other immunocompromised individuals. An earlier clinical trial reported a breakthrough case of wild-type DENV infection with uncomplicated febrile illness in an adolescent vaccinee with onset 4 days after the second vaccination dose [24]. However, to date, there have been no indications of an increased risk of severe dengue disease following vaccination, based on several thousand doses of varying LATDV candidates administered in clinical trials [7,11,27].

Vaccine-associated dengue-like disease can potentially occur as an early or later onset event after vaccination. Early post-vaccination dengue may theoretically result from vaccine viraemia but is unlikely due to the low level of replication of the attenuated viruses. Of more potential concern is enhancement due to infection with wild-type DENV before the full immune response to vaccination has developed or as a later post-vaccination adverse event. Evidence for such risks has not been identified to date in pre-licensure trials of current candidate vaccines.

Later onset post-vaccination dengue might result from immune enhancement following infection by a wild-type DENV in individuals with insufficient protective immunity generated by the primary immunization series or waning immunity over time resulting in susceptibility to one or more wild DENV serotypes. Such late effects may be related to the interval between completed vaccination and subsequent wild-type DENV exposure, the viral load from wild-type DENV infection(s) and the level of immunity at the time of that exposure and, depending on the local epidemiology of dengue infections, could manifest many years post-vaccination [13].

Depending on the specific vaccine construct, other safety considerations may apply, such as viscerotropic disease for yellow fever vaccine chimeric constructs [28].

Only a small percentage of patients with secondary natural infections, estimated as 2–4% in one review [29], develop severe dengue. Moreover, severe disease can occur after primary infection, in the absence of maternally derived antibody, suggesting a complex, multifactorial pathogenesis [30]. In addition to the immune mechanisms for severe disease discussed above, a number of risk factors have been proposed based on the natural history of dengue, as outlined below. The role, if any, of such factors in severe dengue following vaccination are unknown and may justify inclusion in special safety studies. Varying levels of evidence have been observed in different settings, suggesting that specific attention should be given to collecting safety data from a variety of transmission settings and target populations.

**Age:** A higher relative incidence of severe dengue has been reported in infants and younger children and is thought to

be linked to greater permeability of the vascular endothelium [31–33].

**Gender:** Higher degrees of severity and mortality are reported in girls, however it is possible that this is due to differences in health-seeking behaviour [9].

**Genetic factors:** Studies of specific viral and host genotypes suggest different levels of virulence in DENV strains and that host genotype may increase susceptibility to severe disease [9,34].

**Sequence of the infecting serotypes:** The highest risk of severe dengue following secondary infection has been reported with DENV2 [35,36]. However, reported risks of severe disease vary with a different order of serotypes in the primary and secondary infections, suggesting other important risk factors besides the sequence of infecting serotypes.

**Interval between infections:** Data from Cuba suggest that longer intervals between infections result in more severe disease. Following a 1977 DENV1 epidemic, severe dengue and death in subsequent DENV2 epidemics occurred in 5% and 0.1% of infected persons respectively in 1981, and 42% and 2.3% respectively in 1997 [13]. Further evidence from a cohort study in Thailand showed the average time interval between two infections, among subjects with dengue seronegative (hemagglutination inhibition) status at enrolment, was 1.4 years for asymptomatic patients, 1.9 years for dengue fever and 2.6 years for dengue haemorrhagic fever [37].

**Other virological factors:** The potential for selective immune pressure resulting in escape of variant DENV strains is an important consideration. Epidemiological characteristics of dengue do not suggest immune escape by antigenically variant viruses within a serotype in natural human infections and the available experimental data also suggest that all genotypes of a given serotype are neutralized by polyclonal antibodies [38,39]. Nonetheless, ongoing virus surveillance is crucial [4] and the possibility of increased risk of disease associated with mutant DENV strains need further study, including the severity and characteristics of dengue disease that may result. Because of this, DENVs that cause disease post-vaccination should be systematically isolated and sequenced.

Genetic, antigenic and phenotypic diversity exist within each DENV serotype [40]. The level of efficacy that a particular LATDV induces may vary by genotypes and serotypes, and may therefore affect its long-term safety with respect to the risk of enhanced disease. Hence, long-term studies should, whenever possible, examine circulating genotypes, duration of protection and herd effects.

### 3. Methodological considerations

As with other vaccines, key outcomes of interest in the post-licensure evaluation of safety and effectiveness for LATDVs will include (a) serious adverse events (SAEs) too rare (<1 in 1000) for detection with confidence to have been detected in pre-licensure trials; (b) SAEs with a long latency not detected in pre-licensure trials; (c) adverse events in groups who may have been excluded from pre-licensure trials, such as immune-compromised persons; (d) the level of protection against dengue and severe dengue under field conditions; (e) potential waning of protection and the need for boosters; and (f) evidence of herd protection.

Considerations of safety and efficacy are inseparably related for dengue vaccines as severe dengue disease is a theoretical adverse reaction as well as an endpoint against which the vaccines are designed to protect. Based on the deliberations of the consultation, a framework is proposed for the long-term assessment of LATDVs (Table 1). The issues for consideration in the framework are grouped according to pre-licensure and post-licensure vaccine use. Potential safety risks are discussed as early and later post-vaccination events as described above. The phase of vaccine introduction and use (pre-licensure or post-licensure) and respective post-vaccination

windows for early or later adverse events pose different issues for study design. We give special attention to the long-term assessment of immune enhancement of disease.

#### 3.1. General methodological issues

##### 3.1.1. Dengue transmission

The design of long-term studies should take into account the need to assess vaccine performance in a variety of settings with different transmission intensities and the potential impact of vaccine coverage levels on mosquito transmission and herd immunity. Both baseline and longitudinal data on circulating DENV serotypes will be of critical importance for the interpretation of these studies.

##### 3.1.2. Ethical issues

An ethical and logistic issue concerns the inclusion of unvaccinated comparison groups (other than those who refuse vaccination) in studies after a dengue vaccine has been proven efficacious and is licensed. Experts at the consultation took into account that, based on current practices for scaling up new vaccines after first introduction, the first LATDVs are likely to be unavailable for large numbers of individuals for some period after licensure even in endemic countries. Thus, in some settings, unvaccinated “controls” may be available for comparative studies until there is widespread public health use of the vaccine. There might also be an opportunity for a coordinated staggered vaccine introduction.

##### 3.1.3. Ascertaining vaccination status and infection exposure

Accurate ascertainment of vaccination status by dosage and timing (including any vaccination of subjects in control groups during long observation periods) and disease endpoints (particularly severe dengue-like illness) may be challenging for long-term studies. Access to accurate vaccination data through vaccine registries is needed and such registries should be established wherever possible, at least for early introducer countries. Documentation and assessment of post-vaccination asymptomatic exposure to dengue virus is likely to be difficult in the general population. However, for special studies, regular serological testing in a subgroup of vaccinees would be desirable to assess any boosting effects of repeated exposures and for monitoring of non-severe dengue. Dengue surveillance systems and laboratory diagnostic capacities are likely to be of variable quality in countries where the vaccine is introduced and efforts should be made to enhance surveillance. There are strong arguments for establishing a number of sentinel sites, with good surveillance and diagnostic capacities, to support long-term (>5 years) safety studies. Priority should be given to establishing sentinel sites in early introducer countries.

##### 3.1.4. Immune monitoring

Where possible, long-term studies should be designed to obtain samples of sera and peripheral blood mononuclear cells (PBMCs) from participants pre-vaccination, at the end of vaccination and periodically thereafter [20]. This will enable study of asymptomatic infections, correlates of protection, the need for booster doses prompted by declining DENV antibody titres, and of biomarkers for the risk of severe dengue. Such repeated blood sampling is likely to be more feasible in the follow-up of clinical trial subjects than in post-licensure studies. As previously recommended, collected samples should be stored for retrospective analysis of dengue cases [20].

#### 3.2. Issues specific to long-term follow-up of clinical trial subjects

To supplement the WHO guidance on safety monitoring of LATDVs during clinical trials [4,20], the experts at the consultation

**Table 1**  
 Framework for the assessment of live attenuated tetravalent dengue vaccines, including considerations for special long-term studies.

	Pre-licensure phases <sup>a</sup> of dengue vaccine use	Post-licensure phase <sup>a</sup> of dengue vaccine use
Key safety questions (especially those that are more specific to dengue vaccines, including the potential risk of severe dengue post-vaccination)	<p><i>Early post-vaccination events<sup>b</sup></i></p> <ul style="list-style-type: none"> <li>• Risk of post-vaccination dengue-like disease caused by vaccine viraemia</li> <li>• Risk of immune-enhanced dengue caused by wild-type dengue virus (DENV) infection in persons with incomplete protection at inter-dose intervals or before development of full immune response (after the last vaccine dose)</li> </ul> <p><i>Later post-vaccination events<sup>b</sup></i></p> <ul style="list-style-type: none"> <li>• Risk of immune-enhanced dengue from wild-type DENV infection post-vaccination due to insufficient vaccine-induced serotypic immune response or waning immunity, or more virulent serotype infection that mimics severe dengue without contribution of immune enhancement</li> </ul> <p><i>Additional safety issues</i></p> <ul style="list-style-type: none"> <li>• Risk of rare serious adverse events (SAEs), of early or late onset, e.g., viscerotropic or neurotropic disease related to the dengue chimeric vaccine employing a yellow fever vaccine backbone</li> <li>• Risk of other unexpected adverse events</li> </ul>	
Study population(s)	<ul style="list-style-type: none"> <li>• Clinical trial subjects, including flavivirus-naïve and flavivirus-immune individuals</li> <li>• Special populations and situations for assessment of specified safety questions, usually in Phase III trials (e.g., immunocompromised persons, co-morbidities, co-administration with other vaccines)</li> </ul>	<p>The priority study populations may be determined according to the stage and strategy of vaccine introduction</p> <ul style="list-style-type: none"> <li>• <i>Before general public health use:</i> If considered necessary beyond studies in the pre-licensure period, Phase IV studies may be carried out in trial settings (including special populations and situations e.g., immunocompromised persons, co-administration with other vaccines)</li> <li>• <i>At introduction into public health use:</i> Phase IV studies in early introducer countries</li> <li>• <i>During routine or general public health use:</i> Phase IV studies in early introducer or selected countries. (Special studies for late events only if needed<sup>c</sup>)</li> </ul>
Possible study designs	<p>Individually randomized controlled trials (± nested case-control studies)</p> <p><i>Key issues in study design:</i></p> <ul style="list-style-type: none"> <li>• Study designs should enable assessment of protection and risk profile for all 4 serotypes – not necessarily in the same study population</li> <li>• For long-term follow up, there are ethical constraints regarding how long an unvaccinated control group can be maintained once there is substantial evidence of efficacy           <ul style="list-style-type: none"> <li>• Consider possibility to bleed all or most participants at the end of primary vaccination, and periodically thereafter, to study correlates of protection and biomarkers of risk</li> <li>• Studies should assess if and when booster doses should be given</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• <i>Before general public health use:</i> Randomized controlled trials (± nested case-control studies)</li> <li>• <i>At introduction into public health use:</i> Phased introduction, e.g., stepped wedge design, gives opportunity to assess (a) risk profile by time since vaccination and (b) effectiveness/public health impact</li> <li>• <i>During routine or general public health use:</i> Case-control studies or cohort studies. Case-only studies may be appropriate for early adverse events</li> </ul> <p><i>Additional considerations:</i></p> <ul style="list-style-type: none"> <li>• Surveillance for safety signals may be done through routine monitoring in the post-licensure phase</li> <li>• Special studies and/or enhanced detection and virological work up may be required for some specific SAEs and/or signals</li> </ul>

<sup>a</sup> Long-term follow-up of vaccinees in Phase II and Phase III trials may extend to the post-licensure period for a given vaccine.

<sup>b</sup> “Early events” are defined here as adverse events occurring from first vaccination and up to approximately 21 days after the last dose in the primary series. “Later events” are defined as adverse events occurring at any time beyond the period for early events and may extend to many years post-vaccination.

<sup>c</sup> May be considered if long-term studies are not planned with early vaccine introducer countries “at introduction into public health use” or if they are inadequate to provide reliable data.

discussed additional considerations for the assessment of safety. Long-term follow-up of clinical trial subjects beyond the current guideline of 3–5 years post-vaccination, should continue for as long as possible to better describe the safety profile. This should be done whether or not an unvaccinated control group can be maintained. Decisions regarding an unvaccinated arm in

comparative studies post-licensure will require inputs from sponsors, regulatory authorities, ethics committees and data safety monitoring boards. There are precedents for extended follow-up of vaccinated and unvaccinated participants in other studies, beyond the time when short-term efficacy has been established [41–43].



The risks of early and later adverse events will need to be evaluated in clinical trial populations, including subjects who were flavivirus-naïve and flavivirus-immune pre-vaccination. Safety and efficacy may also need to be established in specific population groups potentially at higher risk but excluded from earlier trials (e.g., HIV-infected persons or pregnant women). Some potential risk groups may be too small to justify specific trials (such as persons with certain immunodeficiency syndromes or other clinically significant co-morbidities such as diabetes and sickle cell anaemia, or those receiving immune-modulating drugs), but in these groups SAEs should be monitored through specific assessment protocols.

### 3.3. Possible study designs for long-term safety assessment post-licensure

The selection of appropriate study designs will depend on the stage and strategy of vaccine introduction in a specific setting.

#### 3.3.1. Before general public health use

There may be opportunities for setting up studies before any dengue vaccines are marketed for widespread use in a country, or in the period between the completion of Phase III studies and vaccine licensure. In this period, it may be possible to use randomized controlled studies to assess both effectiveness (including herd protection with cluster randomized designs) and safety. Ethical limitations on maintaining unvaccinated comparison groups may be less challenging under these conditions but are still likely to limit the duration of follow-up [43,44].

#### 3.3.2. At introduction into public health use

Where phased introduction of an LATDV for routine use is being considered, a stepped-wedge design may be possible, in which populations in different geographical areas are vaccinated at different times, allowing those who are unvaccinated at any given stage to serve as controls until they are vaccinated [45]. The stepped-wedge design is generally more acceptable than a randomized controlled trial or a cluster-randomized trial for post-licensure studies. Its usefulness in the context of LATDVs is likely to be limited to the assessment of early post-vaccination events, as it may not be acceptable to maintain unvaccinated comparison groups for long periods.

#### 3.3.3. During routine or general public health use

Methodologically and logistically, case-control studies are the easiest and most feasible option, although ensuring accurate retrospective ascertainment of vaccination status may be challenging and the statistical power of such studies is compromised in situations of high vaccine coverage. Prospective cohort studies have the advantage that vaccine status can be accurately recorded but in some situations there may be an ethical obligation to offer vaccine to unvaccinated controls. Retrospective cohort designs may be possible in settings where accurate records of vaccination and disease outcomes are available, such as through linked computerized databases.

Difficulties in the interpretation of both case-control and cohort studies arise from the non-random allocation of vaccination and the challenges of adjusting adequately for confounding factors (e.g., those at the highest risk of disease might be most (or least) likely to present for vaccination). For some vaccines, case-only studies have proved useful for assessment of potential adverse effects occurring shortly after vaccination [46] and this design may also be considered for early events following dengue vaccination. As with the other designs, case-only studies require reliable ascertainment of vaccination status as well as disease outcomes. Their design relies on a hypothesized window of increased risk following vaccination and thus for LATDVs would be less applicable for assessing

later post-vaccination adverse events for which the period of risk is currently undetermined.

In addition to special studies, the introduction of LATDVs should be used as an opportunity to strengthen routine systems for post-marketing surveillance of adverse events following immunization. This may be foreseen through global or regional activities such as the Global Vaccine Safety Initiative [47]. Some SAEs, such as suspected cases of viscerotropic and neurotropic disease following the yellow fever chimeric vaccine, may require enhanced surveillance and virological work up [28].

## 4. Other considerations

### 4.1. Ascertainment of severe dengue

There are potential challenges with regard to both the definition of dengue disease and classification of case severity, including the availability of diagnostic tools for severity ascertainment (e.g., difficulties with plasma leakage assessment in low-resource settings). Furthermore, there are currently no diagnostic assays to distinguish severe dengue cases due to potential vaccine-associated immune enhancement from those cases due to vaccine failures. Therefore, assessment of risk-benefit will depend upon epidemiological approaches. Cases of severe dengue are more likely to be captured by dengue surveillance than by weak adverse event surveillance systems. Experts at the consultation discussed the adequacy of the current WHO dengue case classification [1] to monitor post-vaccination dengue illness and concluded that further work is warranted to ensure complete, accurate and harmonized classification of cases in the context of monitoring long-term safety and effectiveness of dengue vaccines.

### 4.2. Diagnostics

Various diagnostic tests for dengue infection are available, some of which are able to distinguish different DENV serotypes [48]. Due to molecular differences between wild-type dengue viruses and vaccine viruses, virus sequencing and other assays will be essential to assess the causality of dengue-like illness occurring shortly after vaccination. For example, chimeric yellow fever dengue vaccine viruses can be distinguished from naturally occurring dengue viruses by assays detecting nucleic acid, antigens and antibodies specific to the yellow fever non-structural genes.

Collection and long-term storage of relevant samples from vaccinees (e.g., serum, PBMCs) should be encouraged, as this could greatly facilitate efforts to determine correlates of protection, possible booster needs and potential biomarkers, which may be used in the future to identify vaccinees at risk of dengue disease.

### 4.3. Potential role of modelling

Models to assess the impact of dengue vaccine introduction, based on current concepts of dengue transmission dynamics, may be useful, particularly in the context of assessing potential herd protection [49].

## 5. Conclusions

Current candidate LATDVs in clinical trials appear to have acceptable short-term safety profiles, however their long-term safety has yet to be confirmed. Severe disease due to vaccine failure and vaccine-induced immune enhancement of disease are likely to be indistinguishable in individual vaccinees and benefit-risk assessments will have to rely on epidemiological studies. Both human host and viral factors could theoretically influence vaccine

safety and merit careful evaluation in long-term safety assessments of LATDVs. Randomized controlled study designs, such as in Phase III studies, will provide the most powerful and unbiased datasets to assess these issues. The current WHO guidance for follow-up of trial participants for 3–5 years post-vaccination should give a solid basis for medium-term safety data, particularly if unvaccinated controls can be maintained through this follow-up period. Further follow-up of clinical trial subjects for longer periods is desirable to help establish the duration of protection and the risks of late adverse events. In addition to follow-up in clinical trials, further long-term assessment of LATDVs through special post-licensure studies is needed to support benefit-risk evaluations. Close collaboration between licensing national regulatory authorities, and with respective vaccine sponsors, is recommended for a coordinated approach to the design and implementation of long-term follow-up studies.

In this paper we have outlined methodological issues which support a risk-based approach in the long-term assessment of LATDVs, particularly beyond the follow-up of subjects in pre-licensure clinical trials. There are strong arguments for a coordinated approach to develop a comprehensive and robust post-licensure safety database for dengue vaccines in parallel with their expanded use. Multiple stakeholders, including national public health, surveillance and regulatory authorities; national ethical committees; dengue vaccine developers; academic and clinical experts in the diagnosis, treatment and control of dengue; and international donors will all have roles in this coordinated approach. However, it should be emphasized that not every country that deploys dengue vaccines will necessarily have to conduct studies of the kind outlined above. Rather, what is needed are exemplar studies, supported by systematic dengue surveillance, in carefully selected sites whose outcomes will inform national, regional and global vaccination strategies. As for any other new vaccine, all countries should be aware of possible vaccination risks. National regulatory authorities, particularly in early introducer countries, may consider to make licensing decisions conditional on the conduct of specific post-licensure studies to assess both effectiveness and safety.

Reliable and robust data on the long-term safety of LATDVs will primarily serve to establish a robust safety profile given the theoretical risks. These data will also be critical to identify and manage unsubstantiated safety concerns about severe dengue in vaccinees that could put vaccine programmes at risk.

### Conflicts of interest

AB-E, RE, JF, JH, JTR and JS declare no conflicts of interest. AD has served as a speaker in scientific consultations on dengue vaccines and works through her institution on ongoing dengue vaccine trials sponsored by the US National Institutes of Health. PGS serves on the Independent Data and Monitoring Committee for ongoing dengue vaccine trials being conducted by Sanofi Pasteur.

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