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Review

Live-attenuated tetravalent dengue vaccines: The needs and challenges of post-licensure evaluation of vaccine safety and effectiveness

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ABSTRACT

Since December 2015, the first dengue vaccine has been licensed in several Asian and Latin American countries for protection against disease from all four dengue virus serotypes. While the vaccine demonstrated an overall good safety and efficacy profile in clinical trials, some key research questions remain which make risk-benefit-assessment for some populations difficult. As for any new vaccine, several questions, such as very rare adverse events following immunization, duration of vaccine-induced protection and effectiveness when used in public health programs, will be addressed by post-licensure studies and by data from national surveillance systems after the vaccine has been introduced. However, the complexity of dengue epidemiology, pathogenesis and population immunity, as well as some characteristics of the currently licensed vaccine, and potentially also future, live-attenuated dengue vaccines, poses a challenge for evaluation through existing monitoring systems, especially in low and middle-income countries. Most notable are the different efficacies of the currently licensed vaccine by dengue serostatus at time of first vaccination and by dengue virus serotype, as well as the increased risk of dengue hospitalization among young vaccinated children observed three years after the start of vaccination in one of the trials. Currently, it is unknown if the last phenomenon is restricted to younger ages or could affect also seronegative individuals aged 9 years and older, who are included in the group for whom the vaccine has been licensed. In this paper, we summarize scientific and methodological considerations for public health surveillance and targeted post-licensure studies to address some key research questions related to live-attenuated dengue vaccines. Countries intending to introduce a dengue vaccine should assess their capacities to monitor and evaluate the vaccine's effectiveness and safety and, where appropriate and possible, enhance their surveillance systems accordingly. Targeted studies are needed, especially to better understand the effects of vaccinating seronegative individuals.

Contents

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1. Introduction

With an estimated 96 million clinically apparent infections every year, dengue is the most common mosquito-borne viral disease globally [1]. The highest disease burden is now found in Asia and Latin America with expanding geographic distribution and co-circulation of multiple dengue virus (DENV) serotypes [2]. Four antigenically distinct serotypes (DENV1-4) are recognized and infection with one is generally believed to confer life-long immunity to that serotype (homotypic protection) while cross-immunity to other serotypes (heterotypic protection) persists for one or two years [3,4].

Most DENV-infections are asymptomatic or cause a mild illness, but a small proportion of cases develop severe disease manifested as plasma leakage, bleeding or severe organ involvement. For example, in two large phase 3 trials of the now licensed dengue vaccine carried out in 10 endemic countries, about 10,000 unvaccinated children aged 2–16 years served as controls. Among these children there were 708 virologically-confirmed dengue infections identified in the first 2 years of follow-up (2.9 and 4.6 episodes per 100 person-years in Latin America and Southeast Asia, respectively), of which 30 (4%) were classified as dengue haemorrhagic fever, including 2 (0.3%) with dengue shock syndrome [10]. Multiple epidemiological studies have shown that the risk of developing severe disease is higher after a heterotypic second DENV-infection (estimated 0.5–2%) as compared to the risk following primary infection [5–7]. Antibody-dependent enhancement of infection has been proposed as a possible underlying mechanism [8,9]. The determinants of severe disease include viral as well as human host factors, such as age and ethnicity [8].

Starting in December 2015, the first dengue vaccine (Dengvaxia®, Sanofi-Pasteur) was granted market authorization in several countries in Asia and Latin America. This vaccine is a tetravalent, recombinant, live-attenuated vaccine with a yellow fever 17D vaccine virus backbone, administered in a 3-dose schedule at 6-month intervals. The vaccine has been licensed for individuals aged 9–45 years living in dengue endemic areas, although both upper and lower age limit might vary by license. This vaccine is not intended as a routine vaccination for travellers [11]. Currently, the vaccine is mainly available to the private market, but it has been introduced in subnational public sector programs in Brazil and the Philippines [12]. Two other tetravalent live recombinant vaccines, TV003/TV005 and TDV, are undergoing phase 3 trials (Table 1).

In July 2016, based on recommendations of the Scientific Advisory Group of Experts (SAGE) on Immunization, the World Health Organization (WHO) published a position paper providing guidance for the use of Dengvaxia® [13]. WHO recommends that countries should consider introduction of the vaccine only in geographic settings (national or subnational) where epidemiological data indicate persistent and high intensity of transmission. Prior infection with DENV of any serotype, as measured by seroprevalence, should ideally be 70% or greater in the age-group targeted for vaccination to maximize public health impact and cost-effectiveness [14]. The main reason for this recommendation is the differential performance of Dengvaxia® by dengue serostatus at the time of first vaccination, in terms of vaccine efficacy, and possibly safety [13]. Details of the trial results informing the WHO position are summarized below.

As for any new vaccine, some questions remain unanswered at the time of licensure, which can only be studied once the vaccine is widely used in the population. Accordingly, concerted post-licensure evaluation of a new vaccine will be conducted by the manufacturer according to a risk management plan (RMP), and should also be planned by public health authorities that include the vaccine in their national or subnational immunization program. However, the complexity of dengue epidemiology, pathogenesis and population immunity, as well as some characteristics of the currently licensed dengue vaccine, pose considerable challenges as some research questions cannot be answered simply through using routine surveillance systems and require either enhanced (active) surveillance activities or targeted studies. Foreseeing the need for enhanced country surveillance and targeted studies, considerations for post-licensure evaluation of live-attenuated dengue vaccines have been previously published [15–18]. In light of the results of the phase 3 clinical trials and the WHO/SAGE recommendations, it is appropriate now to refine and prioritize the needs for post-licensure data. Building upon these previous considerations, the background paper of the SAGE work-

Table 1
Dengue vaccine candidates in clinical development (as of August 2017).*

Candidate vaccine name	Manufacturer/ Developer	Vaccine type/platform	Antigen	Adjuvant	Most advanced trial phase
CYD-TDV (Dengvaxia®)	Sanofi Pasteur	Live, recombinant (based on a yellow fever vaccine 17D backbone)	DENV-1–4 prM/E	None	Licensed
TV003 TV005	Butantan Institute	Live, attenuated and recombinant (DENV-2 in DENV-4 backbone)	DENV-1,3,4 whole genome, DENV-2 prM/E	None	Phase III
TDV (also referred to as TAK-003)	Takeda	Live, attenuated and recombinant (DENV-1/3/4 in DENV-2 backbone)	DENV-2 whole genome, DENV-1,3,4 prM/E	None	Phase III
TDENV-PIV	GSK/WRAIR/ Fiocruz	Inactivated	DENV-1–4 whole genome	AS03	Phase II
TDENV-LAV + TDENV-PIV	WRAIR	Live, attenuated and inactivated (heterologous prime-boost)	DENV-1–4 whole genome	Alum (with PIV)	Phase I
V180	Merck	Recombinant subunit (non-VLP)	DENV-1–4 E protein	ISCOMATRIX	Phase I
TVDV	NMRC	DNA	DENV-1–4 prM/E	Vaxfectin	Phase I

* Sources: World Health Organization (WHO) Global Observatory on Health R&D http://www.who.int/research-observatory/monitoring/processes/health_products/en/ and WHO vaccine pipeline tracker http://www.who.int/immunization/research/vaccine_pipeline_tracker_spreadsheet/en/.

ing group on dengue vaccines [19], and discussions during a meeting on "Targeting vaccination and post-licensure studies for the licensed dengue vaccine" organized by WHO in June 2016 [20], we summarize the current scientific background and highlight important post-licensure studies that are feasible for early introducer countries to perform, as well as critical targeted studies that can best be addressed in special research contexts. Second-generation tetravalent live-attenuated dengue vaccines may have different characteristics to the currently licensed vaccine, but the surveillance activities and studies we propose are likely to be relevant for the post-licensure evaluation of future live-attenuated and, possibly, non-live dengue vaccines. We will not discuss the studies that are needed to inform vaccine introduction decisions.

2. Key findings from phase 3 studies

Dengvaxia® has been evaluated in two parallel phase 3 trials in 5 countries in Asia (trial CYD14) and 5 countries in Latin America (trial CYD15) [21,22]. The trial in Asia included 10,275 participants aged 2–14 years and the trial in Latin America 20,869 participants aged 9–16 years. There was a random assignment, in a 2:1 ratio, to the vaccine or the control group. Both trials included active surveillance for dengue for 25 months after the first dose for the primary efficacy endpoint (virologically-confirmed dengue disease) and hospital-based surveillance for a further 4 years for additional safety evaluation. In the hospital-based follow-up period, which is ongoing, the incidence of hospitalization for dengue was monitored to evaluate any potential predisposition in vaccinated persons to more severe disease [23]. Serostatus at baseline was assessed in a subset of trial participants (1983 and 1944 participants in CYD14 and CYD15, respectively). The proportion of children having pre-vaccination antibodies against at least one DENV-serotype increased with age [19] and, among participants aged 9 years or older, was approximately 80% in both trials [21,22].

Based on pooled results from both trials, 3-dose vaccine efficacy across all age-groups in the 25 months after the first dose was 60.3% (95% CI, 55.7–64.5) [23]. Efficacy varied by infecting serotype (higher for DENV-3 and -4), age at time of first dose (65.6% and 44.6% in children aged 9–16 and <9 years, respectively), and was higher for protection against more severe disease (80.8% against dengue hospitalization and 93.2% against severe dengue in children aged 9–16 years) [23]. Independent of age, vaccine efficacy was higher among vaccinees who were dengue seropositive than among those who were seronegative at baseline (70.1%; 95%CI 32.3–87.3 vs. 14.4%; 95%CI -111–63.5 in children aged <9 years, and 81.9%; 95%CI 67.2–90.0 vs. 52.5%; 95%CI 5.9–76.1 in those aged 9–16 years) [23]. There are currently no data on the efficacy of the vaccine against the primary endpoint beyond 25 months after the first vaccine dose, as in this period surveillance was limited to hospitalized cases only. Some data are expected to become available as, later in the trials, active surveillance for the primary endpoint was reinstated [23]. In children ≥9 years, the relative risk of hospitalized dengue, in the period 2 or more years after trial entry, was lower in the vaccinated group, but moved closer to unity as time progressed, suggesting waning protection within a few years post-vaccination [19].

With respect to vaccine safety, the incidence of severe adverse events following immunization (AEFI) was similar in the vaccine and the control group [24]. Since Dengvaxia® is a chimeric vaccine using the yellow fever 17D strain virus as backbone, there was a hypothetical risk of yellow fever vaccine-associated viscerotropic or neurotropic disease [18]. No such adverse reactions were observed during the trials, but the studies lacked sufficient power to exclude risks below 0.1%.

In the 3rd year after the first dose, an interim safety analysis demonstrated a relative risk (RR) of hospitalization for dengue of 0.50 (95% CI, 0.29–0.86), compared to the control group, among those aged 9 years or older at first vaccination. However, in those first vaccinated aged 2–5 years there was a significant excess of dengue hospitalization in the vaccinated group (RR = 7.45; 95%CI 1.15–313.80). This was also the age-group with the lowest pre-vaccination seroprevalence (51%) [21,23]. Among children hospitalized for dengue <9 years of age, there were 12 cases of severe dengue in the vaccine group and none in the control group; no deaths occurred [23]. No difference was observed in the level of viremia and cytokine profile of those vaccinated compared to controls [23].

Since the pre-vaccination serostatus was assessed only in a subset of participants, and the proportion of seronegatives in children aged 9 years and older was small, stratified analysis of the risk for dengue hospitalization or severe dengue by serostatus was not informative. It remains unknown if the differential vaccine performance is related to age, serostatus, or both factors. Several interrelated biologic hypotheses have been discussed [19,23,25,26]. Since the efficacy of Dengvaxia® is moderate, breakthrough infection, including severe disease, occur. One hypothesis for the excess of dengue hospitalization in younger vaccinees is that, in seronegatives, Dengvaxia® mimics a (first) silent primary monovalent or bivalent infection and when heterologous cross-protection wanes, the first wild-type infection acts like a second infection, leading to more severe disease [8,27]. Younger children may be more susceptible to this phenomenon as this group includes a high proportion of subjects who are dengue seronegative. Their immature immune responses to the vaccine may induce antibodies with less affinity, and their vascular physiology is more prone to plasma leakage [19,23]. Since second infections after a natural primary infection can also lead to enhanced disease, the question remains why the risk was significantly higher and not equal in the group of young vaccinees as compared to the control group. This could be explained by the condensed enrolment period in the trial that clustered these children to a first "dengue-like" (vaccine) exposure compared to unvaccinated controls, who would be primed naturally over a longer period [19,23]. The risk for dengue hospitalization among vaccinated participants compared to controls in children first vaccinated aged 2–5 years diminished in the 4th and 5th year of the trial and was not statistically significant (RR = 1.42, 95%CI 0.58–3.99 and RR = 1.50, 95%CI 0.27–15.15, respectively). Although, in the total follow-up period from first vaccine dose an excess risk of hospitalized dengue in children first vaccinated aged 2–5 years was observed, this was not statistically significant (RR = 1.26, 95%CI 0.76–2.13) [19].

An alternative explanation for the findings among young children is that the observed breakthrough infections leading to hospitalization were not analogous to secondary-like infection, but just primary infection following a complete waning of homo- or heterotypic protection or a primary vaccine failure. Since the numbers were small, it cannot be excluded that the apparent increase in risk of hospitalizations in the third year for age-group 2–5 years is just a chance, i.e. type II, statistical error. Nonetheless, this increased risk of dengue hospitalization detected in children <9 years in the third year in one of the clinical trials ultimately led to the current vaccine indication for a minimum start age of 9 years.

3. Key research questions for the licensed vaccine

The manufacturer of Dengvaxia® has developed a RMP, which includes long-term monitoring of ongoing efficacy trials, post-marketing pharmacovigilance and safety studies (e.g. background rates of conditions that can mimic viscerotropism and neurotropism, cohort event monitoring, pregnancy register), effectiveness (e.g. community-based studies to evaluate impact on disease transmission, facility-based studies to evaluate impact on hospital-

Box 1 Outstanding research questions related to live-attenuated tetravalent dengue vaccines.

Safety-related questions

1. Is there a risk of enhanced disease associated with the currently licensed vaccine? And if there is a risk of vaccine-associated enhanced disease:
   - 1.1. What determines the risk (e.g. serostatus, age at time of vaccination, or other factors)?
   - 1.2. Is the risk conferred by the vaccine similar to that conferred by natural infection?
   - 1.3. How does it relate to the number of doses received?
   - 1.4. Does waning of vaccine-induced immunity contribute to any risk and does any risk increase with time since vaccination? Does a booster vaccination mitigate the risk?
   - 1.5. Does immunity to other flaviviruses (induced either by natural infection or vaccination) modify any such risk?
2. What is the incidence of rare severe adverse events (esp. acute viscerotropic or neurotropic disease) and post-vaccination dengue-like illness caused by vaccine viremia?
3. Is the vaccine safe in special risk groups, e.g. immunocompromised subjects and pregnant/lactating women?

Effectiveness and impact-related questions

4. What are the effects of vaccination when the vaccine is included in a public program? In particular:
   - 4.1. What is the overall effect (direct and indirect protection) on the incidence of dengue and hospitalized/severe dengue in the population?
   - 4.2. Does vaccination reduce dengue transmission?
   - 4.3. What is the program impact on the dengue serotype distribution in the area?
   - 4.4. What is the effect on the epidemiology of other flaviviruses
5. What is the vaccine effectiveness under field conditions? In particular, are there differences in the vaccine effectiveness
   - 5.1. against asymptomatic and symptomatic dengue?
   - 5.2. by number of doses, age at vaccination (esp. adults) and serotype? This includes the question if fewer than three doses are needed in any individuals (e.g. in those with pre-existing immunity)?
   - 5.3. by time since vaccination (duration of protection)
   - 5.4. by immune status in respect to other flaviviruses (e.g. yellow fever, Japanese encephalitis and Zika virus)?

Methodological questions

6. Can the serostatus at time of vaccination be retrospectively deduced with diagnostic tests, including in patients with post-vaccination dengue virus infection?
7. In a country with limited resources, what are appropriate study designs to assess dengue vaccine effectiveness?
8. Can methods be devised to distinguish if, in a vaccinated patient with a severe breakthrough infection, the disease was enhanced by the vaccine or developed independently from the vaccine?

ization and severe dengue, and monitoring of potential waning immunity) and additional clinical studies (booster studies, administration to clinical stable HIV+ subjects and co-administration with human papilloma and tetanus-diphtheria-pertussis vaccines) [19].

While comprehensive, this RMP does not seem to address directly the observed vaccine effect in seronegative individuals. In addition, manufacturer-independent studies will be valuable, particularly to assess the effectiveness and safety of the vaccine in large public-funded programs. Key research questions related to live-attenuated tetravalent dengue vaccines, along with activities planned by the manufacturer, are summarized in Box 1. Targeted studies will be needed to answer some, while others could be evaluated through establishing enhanced (active) surveillance activities in selected health facilities or strengthening routine monitoring systems in countries introducing the vaccine in their public immunization program. These research questions aim to fill gaps deemed important for country-level policy-making. Most of the research questions can be grouped into one of the three main areas (Box 1). First, what are the effects of vaccination when the dengue vaccine is included in a public program? Second, what is the vaccine effectiveness under field conditions? And third, does vaccination of seronegative individuals increase the risk for hospitalized or severe dengue? If so, is the risk modified by waning immunity or incomplete vaccination and what is the population-level impact? Mathematical modelling based on the hypothesis of secondary-type breakthrough infections suggests the vaccine might increase the overall incidence of more severe dengue in settings with low virus transmission (i.e. where the risk of two or more DENV-infections is low) [14,18].

We propose key research questions that are relevant for an extended risk-benefit-assessment. Out of the scope of this paper are additional research questions related to dengue vaccines that are relevant and warrant investments. For example, which biomarker(s) can serve as immune correlates of protection or disease enhancement, and do immunological effector mechanisms contribute to clinical protection.

4. Methodological challenges when addressing implementation and research questions

There are several methodological challenges to address these key research questions. First, routine disease surveillance systems mainly provide population-based data to compare disease incidences before and after vaccine introduction to assess the impact of a vaccination program. Based on mainly routine reporting systems, these data are limited by case ascertainment definitions, diagnostic accuracy, heterogeneous outcome and severity criteria, and variable surveillance and reporting intensity. Several studies have shown substantial underreporting in routine surveillance systems [28,29], but as long as the level of underreporting is constant this has little impact on trend analyses. Still, any impact of vaccination might not be detectable at population-level when only a small fraction is vaccinated, when vaccination coverage is suboptimal or if vaccine efficacy is only moderate. Also, natural year-to-year variations in dengue incidence and serotype distribution are substantial and challenge the interpretation of temporal changes in surveillance data.

Table 2
Post-licensure study designs and settings to address key research questions.

Research question	Setting	Options for study design	Comment
Safety-related questions			
Risk of severe dengue by serostatus at vaccination, age-group 9 years and older	Targeted study	Prospective cohort study with serostatus determined pre-vaccination or stored pre-vaccination sera	Large sample size required; oversampling of sero-naïve participants in high transmission settings. Other questions can be addressed in such study, e.g. effect of immunity to other flaviviruses
	(Enhanced hospital-based sentinel surveillance)	Case-control study, if a valid test to retrospectively identify serostatus at time of vaccination is available. Compare severe cases with non-severe cases	Currently no test is validated for this purpose
Risk of severe dengue by age at vaccination, number of doses, time since vaccination	Enhanced hospital-based sentinel surveillance	Case-control study. Compare proportion vaccinated among severe cases vs. non-severe cases, with stratifications by risk factors under consideration	Cohort studies not suitable, since they would require a very large sample size, off-label use, or long study period. Limitation: Cannot adjust for serostatus at time of vaccination
Risk of rare severe adverse events, esp. acute viscerotropic / neurotropic disease & post-vaccination dengue-like illness	Routine national adverse events following immunization (AEFI) surveillance	Observed vs. expected analyses	Expected background rates viscerotropism and neurotropism and conditions that can mimic them need to be established (part of the manufacturer's RMP)
Vaccine safety in special risk groups, e.g. immunocompromised subjects and pregnant/lactating women	Routine national adverse events following immunization (AEFI) surveillance	Observed vs. expected analyses. Comparison of outcomes in the risk group vs. non-risk group	Need to include the information of belonging to a special risk groups as a variable for reporting. Risk of bias in retrospective assessments/passive surveillance
	Targeted studies	Randomized controlled trial for selected groups, e.g. HIV + patients. (Register-based) cohort studies, e.g. pregnancy exposure registry. Comparisons of exposed vs. non-exposed individuals belonging to the risk group; or comparisons of outcome incidences in the risk group vs. non-risk group or published reference rates	Registries need to be established (part of the manufacturer's RMP)
Effectiveness and impact-related questions			
Program impact on disease incidence, impact on virus transmission	Routine disease surveillance system (passive surveillance)	National passive disease surveillance, with before-after analysis, using appropriate statistical methods	Need for standardized surveillance protocol, laboratory confirmation of at least a proportion of cases, inclusion of all age-groups. Need to be established before vaccine introduction. Analysis must consider seasonality and annual fluctuations in disease rates
	Enhanced hospital-based sentinel surveillance	Enhanced surveillance with before-after analysis, using appropriate statistical methods	Need to be established before vaccine introduction
	Targeted study	Studies assessing the risk of dengue in unvaccinated persons in populations with high vaccine coverage. Household studies and studies of the prevalence of infected mosquitoes in setting with varying level of vaccine uptake, including before and after vaccine introduction	
Program impact on serotype distribution	Laboratory-based surveillance	Blood samples collected in outpatient clinics and hospitals to be send to reference laboratory	Reference laboratory for serotyping and genotyping to be identified or established
Vaccine effectiveness by age at vaccination, serotype, number of vaccine doses	Enhanced facility-based sentinel surveillance	Test-negative case-control design (TND) with stratification by risk factors under consideration	Limitation: Cannot adjust for serostatus at time of vaccination. TND not yet validated for the assessment of dengue vaccine effectiveness, for the time being this design should be conducted in parallel to traditional case-control studies
Vaccine effectiveness by dengue serostatus. Effect of immunity to other flaviviruses on dengue vaccine-induced protection	Targeted study	Prospective cohort study with serostatus determined pre-vaccination	Sample size will probably limit the analysis of important co-variates (e.g. age at vaccination, serotype)
Duration of vaccine-induced protection	Enhanced hospital-based sentinel surveillance	Test-negative case-control design (TND) with stratification of vaccine effectiveness estimates by time since vaccination	Limitation: Cannot adjust for serostatus at time of vaccination. TND not yet validated for the assessment of dengue vaccine effectiveness, for the time being this design should be conducted in parallel to traditional case-control studies.
	Targeted study	Prospective cohort study to explore impact of pre-vaccination serostatus on duration of vaccine-induced protection	Long-term follow-up with active surveillance for mild/severe dengue required. Sample size will probably limit the analysis of important covariates (e.g. age at vaccination, serotype, pre-vaccination serostatus)

Second, for safety, spontaneous AEFI reporting systems are generally designed to ascertain events relatively close to the time of vaccination and not to evaluate an increased risk of more severe dengue if this occurs years after the vaccination, depending on the intensity of exposure in the population and waning immunity [30]. Every severe or hospitalized dengue infection in a vaccinated

person would, in theory, require reporting to the AEFI system and trigger further investigations. However, there is currently no diagnostic tool that distinguishes enhanced disease potentially associated with the vaccine from that post-natural infection.

Third, in many countries there are no capacities to link information routinely from existing databases (e.g. disease surveillance, vaccination registries, and AEFI surveillance) because of the lack of trained personal and systematic databases. Many immunization information systems are still paper-based, and in many countries patients also attend private clinics or hospitals that are not connected to immunization and surveillance systems.

Fourth, there is no diagnostic test currently able to distinguish the pre-vaccination serostatus of a dengue-vaccinated individual retrospectively, and routinely assessing this before vaccination is not currently considered feasible in large-scale routine vaccination programs. However, when monitoring vaccine safety within the public surveillance systems or targeted post-licensure studies, knowledge of serostatus at the time of vaccination will be important. This would help determining if an association between pre-vaccination serostatus and post-vaccination enhanced risk of severe disease exist, as well as determining the duration of vaccine-induced protection in pre-vaccination seronegative vs. seropositive individuals.

Fifth, prospective cohort studies with baseline testing for dengue-specific and other flavivirus antibodies are needed. However, such studies would need to be large and include a sufficient number of dengue-naïve participants, and participants would have to be followed up for multiple years to assess duration of vaccine-induced protection and any risk of enhanced disease that might not be manifest for several years.

Finally, overall vaccine effectiveness may vary by population and its ecology (transmission intensities, serotype distribution, pre-existing immunity). Boosting effects from exposure to circulating wild-type DENV and other flaviviruses as well as other flavivirus vaccines (e.g. Japanese encephalitis, yellow fever) may affect the duration of vaccine-induced protection. Therefore, studies from multiple sites with different ecologies and population distributions will be needed.

5. Post-licensure surveillance of vaccine effectiveness and safety

As for any other vaccine introduced in national or subnational immunization programs, surveillance systems should be in place and appropriate to assess if the dengue vaccine's benefits are sufficient to outweigh any risks in the population. Principles that should also be applied for dengue vaccines but might require some strengthening of recording systems include:

a. *Documentation of administered vaccines:* Vaccine coverage monitoring at population-level is important not only to measure immunization program performance and accountability, but to enable the interpretation of vaccine impact and safety. To assess questions on duration of vaccine-induced protection and effectiveness of incomplete vaccination schedules and their potential effect on disease severity during breakthrough infections, individual-level data on dates and number of administered vaccine doses is crucial. National or subnational electronic registries would facilitate vaccination status ascertainment and individualized follow-up as well as linkage to surveillance data.
b. *Disease surveillance:* Every country considering dengue vaccine introduction should consider collecting disease surveillance data before and after vaccine introduction to aid assessment of vaccination impact at population-level. However, high-quality surveillance is needed for this purpose. Surveillance must be based on internationally-accepted case definitions with classifications into probable and laboratory-confirmed cases, include ideally all age-groups as well as case-based information on age, location, times, laboratory confirmation and disease severity including death. With the emergence of epidemics of other arboviruses such as Zika and Chikungunya, the syndromic approach that is being used in many countries for dengue diagnosis has lost most of its validity. Despite the above-described challenges in interpreting surveillance data, this might constitute the only source to estimate vaccine impact in countries that lack sufficient resources to implement enhanced sentinel-surveillance or targeted studies. However, vaccine introduction may be an incentive to strengthen surveillance systems to better define the local dengue epidemiology as dengue becomes a vaccine preventable disease. Several studies have demonstrated that enhancements in laboratory, sentinel-based surveillance, and trained and motivated personnel contributed to improvements in dengue case reporting [28].
c. *AEFI surveillance:* Spontaneous reporting of AEFI is important to identify rare or unexpected adverse events potentially associated with vaccination. Outcomes like viscerotropic or neurotropic disease and hospitalizations for dengue-like illness should be reported and linked to immunization records. The manufacturer's RMP includes the assessment of background rates of conditions that can mimic viscerotropic or neurotropic disease to evaluate changes in their rates following vaccine introduction [19]. Attempts should be made to address the possible risk of vaccine-associated enhanced disease in hospital-based sentinel-surveillance systems [18].
d. *Virological surveillance:* Routine monitoring of circulating DENV sero- and genotypes is important to assess if there is any effect on the serotype mixture [31]. Where possible, DENV derived from post-vaccination cases should be sequenced. In addition, the circulation of vaccine-derived strains should, if possible, also be monitored, but seems unlikely to occur [32,33].

6. Targeted studies to assess vaccine effectiveness and safety

Post-licensure studies to address key research questions are listed in Table 2. As highlighted in the table, the availability of a diagnostic test that can retrospectively identify in a dengue-vaccinated patient their pre-vaccination serostatus would greatly facilitate addressing several research questions. It has been suggested that the presence of anti-DENV envelope IgG in the absence of dengue NS1 IgG antibodies in late convalescent sera, characterizes a first DENV infection on a background of Dengvaxia® yellow fever vaccine–derived immunity in a dengue seronegative person at baseline [25]. However, these tests are not commercially available and not yet validated for this purpose.

The test-negative design (TND) has been proposed for post-licensure assessment of vaccine effectiveness. This case-control design is an efficient and relatively inexpensive epidemiologic method that can easily be embedded in a surveillance program where patients are recruited prospectively through participating ambulatory care clinics or hospitals [34]. The TND has been extensively applied for the evaluation of several vaccines (e.g. influenza and rotavirus) [34–36], and could also be used to monitor dengue vaccine performance after introduction, by comparing the vaccination status of persons presenting with febrile illness, who are shown to either have or not have dengue. Other methods

can be applied which utilize cases ascertained in active sentinel surveillance systems. For example, traditional case-control, case-coverage or case-cohort analyses. The TND offers advantage over traditional case-control designs by reducing the possibility of differential health-care-seeking behaviors among cases and non-cases [34]. However, the TND's validity has not been fully explored in vector-borne infections like dengue. Such validation is a priority.

There are a small number of study methodologies and settings through which key research questions can be addressed (Table 2).

(a) Studies that can be performed within the context of a public health program

These include enhanced facility- or hospital-based surveillance studies which should be considered by early introducer countries to demonstrate the benefits and risks of dengue vaccination in their population. These studies can be implemented in sentinel hospitals with good diagnostic capacities (dengue PCR and NS1 antigen test) for at least 5 years following vaccine introduction. The impact on dengue hospitalizations can be measured, vaccine effectiveness against dengue hospitalization and duration of protection can be assessed using the TND method, and long-term safety can be evaluated through case-control studies. Such studies might examine risk factors for severe disease, including vaccination timing and age.

(b) Studies that require special capacities and resources

Due to the limitations of existing surveillance systems and diagnostic tools, there is a need for targeted studies conducted in selected settings. These include:

- Prospective cohort studies with assessment of the serostatus prior to vaccination and, if possible, periodically thereafter, to evaluate any relation between antibody levels and vaccine effectiveness and safety and monitor for waning immunity or boosting by asymptomatic DENV infections. Additional research questions can be potentially addressed in such prospective studies, such as the impact of immunity to other flaviviruses (e.g. Japanese encephalitis or Zika virus) on effectiveness and safety outcomes, the validity of future assays that can retrospectively distinguish the dengue serostatus at time of vaccination, or better correlates of protection and biomarkers for the risk of severe disease as well as the role of T cell immunity.
- Studies that measure any impact on virus transmission would also be valuable. For example, assessing whether the risk of dengue is reduced in unvaccinated persons in populations with high vaccine coverage.
- More advanced study designs, such as vaccine introduction using a stepped-wedge design or cluster randomized controlled trials, would provide an opportunity to measure indirect vaccine effects and overall effectiveness. However, such designs are complex and require substantial resources.

7. Conclusion

As the first live-attenuated dengue vaccines are introduced in larger populations, several unresolved research questions related to their performance need to be answered. While some are common to all vaccines due to the limited number of participants in pre-licensure clinical development programs, others arose from phase 3 studies that unveiled the complexity of dengue as a preventable vector-borne infection. The current manufacturers' RMP contains a list of activities, including the extension of follow up of participants in the phase 3 trials, which will help to address some of these key research questions. However, as countries introduce the vaccine, they are strongly encouraged to conduct their own post-licensure monitoring and evaluation, which requires planning and potentially strengthening vaccine surveillance and immunization registry systems. Capacities for disease detection, reporting and the assessment of long-term safety need reinforcement, for example, though hospital-based sentinel surveillance. Development and validation of suitable study methods, such as TND, as well as diagnostic tools to retrospectively identify the pre-vaccination serostatus are priorities. One important question for vaccine introduction remaining to be answered and likely will not be tackled by traditional or enhanced surveillance is the effects of vaccinating seronegative individuals, both over time and with respect to age. Targeted studies are needed on this. All entities that collect data on vaccine effectiveness and safety are encouraged to share with the scientific and public community information on the methods and the results generated through these activities to improve informed decision-making at the individual and population level.

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Conflict of interest

OW, KV, ALL, HT and JH declare no conflicts of interest. EJA has received research grants from Takeda Vaccines Inc. and Glaxo-Smith Kline. EJAL has received research grants and travel expenses from Sanofi Pasteur, and has also been an employee of Institute Butantan. IML worked on a project that was partially funded by Sanofi Pasteur. PGS is a member of the Independent Data and Monitoring Committee for the trials of the Sanofi Pasteur dengue vaccine. IKY is the director of the Global Dengue and Aedes-transmitted Diseases Consortium (GDAC) which has received unrestricted grants from Sanofi Pasteur and Takeda Vaccines Inc.

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