In depth examination of important issues for the development and introduction of DENGUE vaccines

Dengue Vaccine Development

Lewis Markoff

Dengue fever (DF) is an acute, self-limited febrile illness characterized by fever, rash, severe myalgia, and occasionally bleeding, and caused by any one of four antigenically distinct viruses, referred to as dengue virus serotypes 1-4 (DENV1-4). In a small minority of patients, a more severe and prolonged form of disease develops, characterized by capillary leakage and leading to severe bleeding and/or hypovolemia with shock. This clinical entity is known as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DHF/DSS may be fatal if proper supportive therapy is not provided in a timely fashion. There are also serious forms of DF that may be life-threatening but which do not fit formal WHO or PAHO criteria for the diagnosis of DHF/DSS. Dengue viruses are transmitted to humans by Aedes mosquitoes, especially Aegypti and Albopictus species. There is no human to human spread. Humans are the major animal host for the virus, although monkeys serve as an alternative host in a sylvan life cycle.
Key Messages

• Dengue is a mosquito vector-borne disease caused by any one of four serotypes of dengue virus. Dengue Fever and its more severe complications represent a huge burden of disease in tropical and subtropical countries.

• Infection with a single serotype of dengue virus confers lifelong protection against that serotype, via induction of virus-neutralizing antibodies and immune memory. Dengue serotype cross-reactive, non-neutralizing antibodies are also elicited, which play a key role in antibody dependent enhancement (ADE) of dengue disease after subsequent "secondary" dengue infections.

• There are no licensed dengue vaccines. Among candidates, live attenuated tetravalent vaccines are in the most advanced stages of development.

• The WHO has published guidelines for the use of dengue vaccines in endemic areas (references within). These guidelines ought to be consulted by vaccine developers before proceeding to plan clinical trials.

• For clinical trials, the standard definitions of phase 1, phase 2, and phase 3 apply to dengue vaccines. Phase 1 and early phase 2 trials will evaluate the monovalent components of a tetravalent vaccine in nonendemic areas. Phase 3 trials will of necessity be conducted in endemic areas using tetravalent formulations.

• The size of phase 3 trials will be determined by the number of subjects needed to show safety and efficacy. A typical safety database would include 3000 to 5000 subjects, cumulative from all phases. The efficacy level of a novel dengue vaccine would probably be set at 70 to 80% for the purposes of study design.

• The primary endpoint for a pivotal efficacy trial will likely be prevention of "dengue", as defined by the occurrence of fever and viremia, caused by any one of the four serotypes of virus.

• Secondary endpoints of a phase 3 trial could include prevention of dengue caused by a particular serotype, prevention of "severe" dengue, and other.

• Since administration of a vaccine could induce a predisposition to severe forms of dengue in vaccinees, as vaccine-induced antibody titers wane, phase 3 trial plans need to account for long term follow-up of at least a cohort of subjects. Three to five years of follow-up is recommended.

• The humoral immune response should be documented in a cohort of subjects, with a view to establishing a surrogate or correlate of vaccine efficacy during the long term follow-up period. A plaque reduction neutralization titer with a 50% endpoint (PRNT50) is recommended for this purpose. There may also be some value in banking PBMCs for future studies.

• Additional clinical data regarding late-occurring adverse events, the relationship of antibody titers to incidence of infection, and the efficacy of the vaccine against different dengue serotypes could be collected in phase 4 studies, to determine a correlate of protection and to determine whether vaccination predisposes to severe disease at late times.

Background

DENVs cause an estimated 100 million cases of DF and more than 500,000 cases of DHF/DSS per year worldwide, in recent estimates. More than 2.5 billion people are at risk. At present there are no licensed vaccines to prevent dengue, and no effective anti-viral drugs are available for treatment. However, several different live, attenuated vaccines are in clinical trials. Vaccines based on killed virus, plasmid DNAs, and purified dengue antigens are also under development.

Dengue viruses are members of the genus flavivirus, nearly all of which are arthropod-borne. Flaviviruses are lipid-enveloped and contain an RNA genome that encodes three structural proteins: envelope, pre-membrane, and capsid, and at least seven non-structural (NS) proteins. The envelope protein is glycosylated and inserted in the lipid outer coat by its carboxy-terminal transmembrane domain. Its amino-terminal ectodomain form dimers that coat the virus surface. The envelope protein serves to attach virus to cells and also contains a fusion activity required for subsequent entry into cells. The humoral immune response to dengue is primarily directed against the envelope, pre-membrane, and certain NS proteins. Dengue infection also elicits a broad cytotoxic and helper T cell response directed against epitopes in NS proteins primarily and in the envelope as well. The nature and
The mechanism for "antibody-dependent enhancement" (ADE) of dengue disease severity has been studied for more than two decades. It is not possible here to review all the work that has been done in an effort to understand the phenomenon on a molecular level, but it is probable that the process is initiated when non-neutralizing or sub-neutralizing concentrations of envelope-specific antibodies present in immune subjects enhance infection of cells of the monocyte/macrophage lineage via binding of antibody/virus complexes to Fc receptors on those cell types. This both enhances virus replication per se and stimulates induction and release of various cytokines and chemokines and perhaps virus-coded proteins that are likely to be causes of capillary leakage\textsuperscript{14,16,19,20}. Although this is the accepted scenario, the precise mechanism of ADE on the cellular level remains unclear. 

Primary dengue infections may be enhanced in infants borne of dengue-immune mothers. Maternally acquired antibodies protect such infants in the first six months of life. However, as those passively acquired antibodies diminish in titer between months six through nine, infants are at increased risk for DHF/DSS as a consequence of a primary dengue infection, a phenomenon that is rarely seen in older children and adults. After nine months of age or after maternally acquired antibody titers have further waned, the risk for primary DHF/DSS diminishes accordingly, and the risk for DHF/DSS in older children, as in adults, is related to the incidence of secondary infections. Other data suggest that infants with DHF/DSS exhibit a unique cytokine profile as compared to that seen in subjects with DHF/DSS due to secondary infections\textsuperscript{13,16,17,18}.

The significance of this response in the development of and protection from disease states has been well studied, and it is clear from available data that a humoral response in the form of virus neutralizing antibodies that recognize envelope is sufficient to protect against illness\textsuperscript{11-14}. Infection with any one of the four DENV serotypes is thought to lead to lifelong protection against disease caused by that serotype, primarily via the induction of envelope-specific virus neutralizing antibodies and probably a memory T cell response\textsuperscript{11}. Cross-protection against serotypes other than that of the primary infecting virus is also induced but persists for only several months post-infection\textsuperscript{15}. After waning of the cross-protective response, the previously infected individual has an increased likelihood of developing DHF/DSS when experiencing a "secondary" infection (i.e., infection by a heterologous serotype of DENV)\textsuperscript{13,14,16}. 

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Problems associated with dengue vaccine development

Need for a tetravalent vaccine. For reasons outlined above, there is a consensus of opinion that any dengue vaccine intended for use in endemic areas should provide protection against all four serotypes of virus simultaneously, to avoid the risk that vaccinees immunized against only one or some of the serotypes might later be subject to severe forms of dengue as a consequence of a secondary infection\(^8,9\). Preliminary clinical data emanating from trials of live tetravalent vaccines indicates that inter-serotypic interference, which abrogates the development of a true tetravalent response, is commonly encountered\(^{21,22}\). Interference may take place either at the level of infection by each of the viruses in a tetravalent mixture, if one or more serotypes directly inhibits or outcompetes the replication of the others at the cellular level, or at the level of the immune system. One strategy that has been used with some success to combat the problem of interference among live vaccines is to alter the relative doses of each of the vaccine viruses in the tetravalent mixture\(^{23}\). The interference phenomenon has not been reliably observed in animal models (see below), and interference among serotypes has been qualitatively different for each of the candidate tetravalent vaccines. Thus the doses of the individual serotypes of vaccine virus in tetravalent mixtures have been determined by small scale clinical trials for each separate product currently under development. It is possible that the interference problem may be mitigated for second generation vaccines that do not need to replicate in order to induce an immune response.

Animal models. Vaccine testing and development is greatly facilitated by the ability to model the disease state in animals. Unfortunately, there is no ideal animal model for dengue disease. Neonatal mice are susceptible by the intracranial route and can be induced to
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lack of an innate immune response; the innate response or lack of same is thought to play an important role in determining the outcome of dengue infection in humans. Monkeys, especially rhesus macaques, develop viremia in response to an infectious dose of dengue delivered peripherally. However, even with human virulent wildtype viruses the levels of viremia are relatively low, and viremia is short-lived. Nevertheless, candidate live virus vaccines have been selected for clinical trial based on a reduction in the duration and intensity of viremia, compared to that achieved by wild-type parent viruses (see for example 33-35). There have been exceptions to the association between a reduction in viremia in monkeys and attenuation in humans. However, this model has some merit as a screening procedure before proceeding to clinical trials and has been universally used in the pre-clinical phase of live vaccine development. Neither genetically normal mice nor monkeys reliably develop the physiological manifestations of systemic dengue infection typically seen in humans, e.g., thrombocytopenia, rash, fever, or evidence of mild hepatitis in the form of enzyme elevations. In summary, the available animal models are not completely satisfactory for predicting the level of attenuation of candidate live vaccines or for predicting immunogenicity and protective efficacy,
although testing of vaccines in mice and/or monkeys is an important aspect of pre-clinical development. Therefore, there is for now at least a degree of uncertainty regarding the potential utility of any dengue vaccine that can only ultimately be resolved in clinical trials.

**Surrogate of efficacy.** While it is generally agreed that virus neutralizing antibodies are sufficient for protection against dengue disease\(^{36}\), there is no agreement on the concentration of such antibodies in blood that is necessary to effect protection. Neutralizing antibodies are classically detected and titered in an in vitro assay in which a constant number of plaque-forming units (pfu) of virus is incubated with serial dilutions of sera. The virus/serum mixtures are then used to infect cell culture monolayers, typically consisting of monkey kidney cells, and the reduction in pfu effected by progressively diluted serum samples is determined by counting the resulting plaques and comparing the results to controls. This is the plaque reduction neutralization test (PRNT). For statistical reasons, a 50% endpoint is used to determine titer (PRNT50). A recent publication provides guidelines for performing this assay on human sera\(^{37}\). Whereas a PRNT50 of 1:10 or less (i.e., the lower limits of detection of the PRNT) has been accepted as a surrogate of efficacy for vaccines directed against diseases caused by the flaviviruses Japanese Encephalitis and Yellow Fever\(^{28,39}\), available data suggest that titers of dengue neutralizing antibodies necessary for protection may need to be well above the minimum that can be detected in vitro and that protective titers may be different for different serotypes\(^{40}\). Developers and regulators anticipate that the first phase 3 trials of live dengue vaccines will provide sufficient data to establish a surrogate of efficacy using PRNT50, but there is no guarantee of this outcome. Establishment of a surrogate of efficacy would greatly simplify the approval process for second generation vaccines.

**In vitro correlates of attenuation.** This problem specifically affects live vaccine development. Attempts have been made to select candidate vaccine strains using the in vitro phenotypes of temperature-sensitivity and host-range restriction. Unfortunately, there are no in vitro phenotypes which reliably predict attenuation of DENVs in
humans, based on current information. However, most live dengue vaccines do exhibit properties in vitro that distinguish them from their respective wildtype parent viruses, and these properties need to be stable during the vaccine manufacturing process and during storage of the product at all times prior to administra-
tion. One highly desirable phenotype of any live vaccine is a reduction in infectiousness for mosquitoes, to reduce the remote risk of reversion and subsequent spread of revertant vaccine virus in endemic areas.

### Potential use of dengue vaccines

Clinical trial design is heavily influenced by assumptions regarding the use of the vaccines in practice in different epidemiological settings. Among other considerations, vaccination strategies depend upon the intensity and duration of protection elicited by the vaccine, the age of subjects most at risk for disease, and the incidence or prevalence of the disease. Some plausible dengue vaccine applications are as follows:

**(i) Routine immunizations.** This may be warranted for dengue endemic areas, e.g., Southeast Asia and the Western Pacific, and for regions with frequent epidemics, e.g., Mexico and parts of Central and South America and the Caribbean. If a dengue vaccine is going to become part of a routine immunization regimen for all children, clinical trials will have to be done to determine whether the dengue vaccine interferes with childhood vaccines already in use in that Locale.

**(ii) Catch-up campaigns.** If routine immunization of infants or young children is adopted as a vaccination strategy, catch-up campaigns could target older children and adults in these countries.

**(iii) Management of dengue epidemics.** Vaccine could in theory be used to interrupt virus transmission during an epidemic, if induction of protection is sufficiently rapid, and if the vaccine is known to be safe and effective for pregnant females and immunocompromised individuals.

**(iv) Travelers.** A dengue vaccine could provide protection to non-immune visitors to dengue endemic areas. Travelers would include tourists, seasonal laborers, and military personnel, among others who might also be put at temporary risk of exposure.
Clinical trial design

Guidelines and customs regarding the conduct of clinical trials of dengue vaccines will inevitably evolve based on the results of studies that are ongoing or will soon be conducted. In addition, current guidelines are based on the perceived problems related to live vaccines. The reader is referred to the WHO "Guidelines for the Clinical Evaluation of Dengue Vaccines in Endemic Areas" for a comprehensive review of the current consensus. In addition, developers should consult the recommendations of regulatory authorities at the country level before proceeding (for example, 43).

**Phase I trials.** The purpose of the phase I trial is to evaluate safety and tolerability in a very small number of subjects, usually adults, as this represents the first use of the product in humans. For live vaccines, information on the frequency and severity of local reactions at the site of injection and of systemic effects, such as fever and malaise is documented. Viremia caused by a live vaccine may also be monitored. Because of the small size of this type of study, only very commonly occurring adverse events can be documented, but when significant safety problems are detected at this level, the information can be used in making decisions regarding the advisability of future studies. Phase I can also be used to establish the dose of vaccine necessary for induction of an immune response, as measured by a PRNT50 (see above). Typically, each monovalent component of current live tetravalent vaccines that have already passed phase I was tested separately in phase 1, in order to establish the safety and immunogenicity of each prior to combining vaccines in a tetravalent mixture. Phase I trials have been conducted in dengue-naïve subjects residing in countries where dengue does not occur. This is done to avoid putting vaccinees at risk for severe dengue, because such individuals will at best develop immunity against only one serotype as a consequence of their participation in the trial. In addition, it is desirable to test live vaccines initially in non-immunes in order to reveal major safety issues that might not occur in previously immune subjects. When tetravalent vaccines are under test, it may be acceptable to conduct trials in endemic areas.
**Phase II trials.** Phase II trials are designed to expand data on safety and immunogenicity, and may also be used to evaluate dose-response. Typically, the numbers of subjects in phase II trials is several times larger than in phase I. Phase II is very flexible. For example, it can be used to bridge data obtained in adults to adolescents and young children or to establish the optimal ratio of monovalent vaccines in the tetravalent mixture. It is conceivable that monovalent vaccines will need to be studied in phase II, but tetravalent formulations of monovalent products that were studied in phase I could also be introduced in phase II. This is a complex question that has been and will be resolved on the country level. In the US, tetravalent formulations have been studied under Investigational New Drug applications that are separate from those afforded to the relevant monovalent products. In the latter part of phase II, studies can be done in endemic areas, so that safety and immunogenicity data obtained from immune subjects that will be relevant to phase III study design can be obtained. As for phase III studies (see below), phase II studies done in endemic areas will have to be accompanied by longterm follow-up of vaccinees for safety, in order to detect whether vaccination alters the susceptibility to severe forms of DF and DHF/DSS. Regulatory authorities seem to agree that three to five years of follow-up is appropriate.

**Phase III trials.** In a phase III trial, final proof of safety and efficacy is sought. Vaccine efficacy (VE) is calculated according to the following equation\(^3^6\): \(\%VE = 100 \times (1 - r_1/r_0)\), where \(r_1\) is defined as the incidence rate in the dengue vaccine group, and \(r_0\) is defined as the incidence rate in the control group.

Most regulatory authorities, including the US FDA, do not mandate any particular level of efficacy for licensure of a novel vaccine, but it is customary to design phase III studies in anticipation of at least 70% or 80% efficacy, especially since the required number of subjects per arm of the study is inversely proportional to the expected efficacy, in order to meet required confidence intervals.
Efficacy trials obviously must be conducted in an endemic area. For this reason, only tetravalent formulations will be tested in phase III. Phase III trials should be conducted in a double-blind fashion. A control arm could involve use of a placebo or of a standard vaccine. Allocation of subjects to vaccine vs control arms of the study should be randomized by any one of many standard computer-based methods. Developers need to have epidemiologic data regarding the seasonal or yearly incidence of dengue in the locale chosen for phase III, and some data regarding the prevalence of one serotype vs the others over time is also advisable. Such information is essential in order to calculate the number of subjects that must be entered into the vaccine and control arms of the study, so that the study can show efficacy within confidence intervals and with sufficient statistical power to satisfy regulatory authorities. An additional confounding factor in phase III trial design for a dengue vaccine is the fact that in endemic areas, mosquito abatement campaigns might be in effect during the time frame chosen for the study. Such a campaign would likely have an effect on the incidence of disease, and developers would have to account for that possible effect in determining the number of individuals that need to be included in each arm of the study.

A phase III trial could be conducted in two geographically proximate communities, where one community receives the vaccine and the other receives placebo or a control vaccine, or it could be conducted within a single community. The WHO Guidelines recommend the latter approach for several reasons:

(i) Dengue transmission is often quite focal even within a community, so over the course of the study the incidence of disease might be quite different between two adjacent communities.

(ii) Vaccination per se of one community could reduce the risk for dengue in that community by attenuating virus spread via mosquitoes, vs the control community.

(iii) The risk for accidental bias is smaller with comparisons between individuals, because the units of randomization are more numerous and easier to stratify or match by exposure.

A phase III trial could be designed to prove efficacy for preventing any of the several forms of dengue, ranging from uncomplicated DF to DHF/DSS. The choice of endpoints is critically important, because these disease entities will have very different incidence rates, and scoring for the more rare events necessitates proportionately larger
numbers of subjects in order to prove efficacy with statistical certainty. Decisions regarding a choice of primary endpoint should also be guided by knowledge of the epidemiology of dengue, which suggests that in endemic areas the prevalent serotype and the number of different serotypes circulating in a given "dengue season" is impossible to predict from year to year, so it is unwise to target a particular serotype or to expect that efficacy against all four serotypes would be demonstrated during the course of the initial study, which is likely to last about a year. Accordingly, the WHO Guidelines suggest as a primary endpoint that phase 3 trials should be designed to prevent "dengue", as defined by a fever of two or three days duration in a subject who can be proven to be infected by any serotype of dengue virus, using in vitro culture, an assay for dengue antigenemia, or detection of dengue genomes in blood using reverse-transcription and the polymerase chain reaction (RT-PCR). Thus the indication for use of the vaccine could be simply stated as "to prevent dengue".

Serology using a single serum sample or acute and convalescent samples is problematic in diagnosing acute dengue, because of the cross-reactivity of antibodies among serotypes and among flaviviruses, in areas where dengue is not the only flavivirus commonly encountered, and because many vaccinees will have previously experienced dengue infections. Although IgM against dengue is presumptive evidence of a primary infection, the response may be blunted or abrogated in vaccinees, due to the very fact of their having been vaccinated.

Establishing secondary endpoints for a phase 3 trial is a good way to obtain a broad descriptive picture of the vaccine effect. Data thus obtained need not necessarily lead to statistical significance.

Secondary endpoints could include:

(i) Efficacy against each of the four serotypes, which is only likely to be demonstrated after several years of observation of the original cohort of vaccinees (see below)

(ii) Efficacy after partial immunization, in the event that the vaccine is to be administered as a 2- or 3-dose Regimen

(iii) Effect of vaccination on the number of hospitalizations for laboratory-confirmed cases of dengue i.e. prevention of severe dengue, and

(iv) Efficacy against ‘possible’ or ‘probable’ dengue disease (to be defined in the study plan)
The study plan must posit acceptable mechanisms for detecting dengue in a (large) cohort of subjects residing in the community under study during phase III. Mechanisms available to meet the goal of rapid detection include periodic home visits or telephone calls by study monitors or instructions to vaccinees to contact study monitors at the first sign of fever. In the latter case, vaccinees and controls would be provided with thermometers and instructed in their use. When fever in a trial participant is reported, it should be documented and blood should be drawn immediately, as viremia and fever correlate with each other in dengue⁴⁴. A laboratory facility has to be readily available for the duration of the study to facilitate proper and rapid handling of all such specimens. Hospitals serving the community also need to be on alert to report emergency room visits and/or admissions of subjects participating in either the vaccine or control arm of the study, to obtain the requisite blood sample necessary to define a case and also to document serious cases of dengue, under the double-blind conditions. The study plan would have to establish formal clinical definitions of "severe dengue" (meaning complicated cases of DF that do not meet the formal definitions of DHF and DSS), DHF, and DSS, especially if prevention of severe dengue is chosen as a secondary endpoint, which is advisable.

Besides proving efficacy, the additional goals of a phase III trial are to accumulate evidence for safety and immunogenicity that can be added to safety data obtained in phase I and II trials in order to satisfy regulatory authorities. Therefore consistency in methodology for evaluating safety throughout development is recommended. A typical goal of safety data is to detect adverse events that occur with an incidence rate of one per thousand vaccinees. To do so with sufficient statistical power requires the evaluation of 3,000 to 5,000 vaccinees in the course of clinical trials. Since the phase III trial may be much larger than 5,000 in terms of numbers of subjects in the two arms, a subset of the population could be selected for detailed safety follow-up. If trials are done exclusively in an adult population, a similar number of adolescents and children need to be evaluated for safety in a separate study, which could be done post-licensure. However, it is quite likely that licensure for use in children as well as adults will be sought for the first dengue vaccines. It is not possible here to review all aspects of safety evaluation, but adverse events are generally treated as either local (e.g., injection site pain, swelling, and redness) or systemic (e.g., fever, malaise, weakness, etc.) Diary cards are sometimes used for days 1 to 21 post-vaccination, so that competent vaccinees
can keep a record of their signs and symptoms. Serious adverse events need to be followed up to final outcome and described in detail in the study report. As for any clinical trial, a Monitoring Board needs to be in place, and strict rules for stopping the study need to be stated and agreed to in advance.

A subset of several hundred of subjects in both arms of the study should be followed for immunogenicity, with samples taken pre-vaccination and at various times thereafter. A virus neutralization assay is recommended to assess immunity with respect to each of the four serotypes of DENV. Because of the large number of pre- and post-vaccination samples that will have to be processed, it may not be practical to employ the classical PRNT described above. Therefore, various high throughput assays are under development. Any of these would have to be standardized and validated against the classical assay. The WHO Guidelines suggest that peripheral blood mononuclear cells (PBMC) should also be collected from at least a subset of the immunogenicity cohort in a phase III trial for future assay of cell-mediated immunity (CMI). These samples would in theory be stored until a decision is made regarding what CMI assays are appropriate. Such a decision might be governed by the nature of unexpected adverse events related to vaccination or a desire to assess immune memory or the cytotoxic T cell response at times after neutralizing antibody titers may wane.

Long term safety follow-up of subjects that participate in phase II and III trials of dengue vaccines is strongly recommended. This should be done:

1. To evaluate the risk that waning vaccine-induced humoral immunity will predispose vaccinees to develop serious dengue infections at late times post-vaccination, and

2. To establish vaccine efficacy eventually against all four DENV serotypes or at least to establish efficacy against serotypes other than those that may circulate during the initial phase of the study (which may be defined as six months post the last dose of vaccine or one year from the start of the study or one dengue season, where vaccination was carried out prior to the start of the season).

As previously noted for phase II trials that might be conducted in endemic areas, three to five years is deemed to be a suitable duration for the additional follow-up period. It is possible that a vaccine could be approved for licensure based on evidence available after the initial phase of the study (see above for definition),
despite the requirement for ongoing follow-up of subjects who participate in phase III, but this will require breaking of the double-blind status of the study. This is an important issue for regulatory authorities to decide. Monitoring subjects for up to five years will require maintenance of an efficient detection and evaluation system for fever of unknown origin and for hospitalizations within the community chosen for phase III. It will also require the continuing participation of study monitors and local hospitals, but it will also likely depend upon the active cooperation of study participants. Particular attention must be paid to the maintenance of an efficient surveillance system for the detection of severe dengue, especially during long term follow-up.

**Phase IV trials.** It is common practice to continue to monitor the effectiveness and safety of a novel vaccine post-licensure. This is known as "phase IV". The exact nature of phase 4 studies that will be done in connection with licensing a dengue vaccine will ultimately be determined by regulatory authorities in conjunction with sponsors. Phase IV studies are routinely conducted to confirm vaccine safety, in a general sense. The goal is to detect vaccine-related adverse events that would not have been detected among the 3,000 to 5,000 subjects evaluated during the approval process, so much larger sample sizes are in order. In addition, it will be advisable to study the potential of a novel dengue vaccine to interfere with routine childhood immunizations, in endemic areas where an effective dengue vaccine might be routinely used in children. It is also likely that a phase IV study will be requested in order to continue the effort initiated in phase III, to establish efficacy of the vaccine against all four serotypes and to determine whether vaccinees are predisposed to severe dengue by the fact of their having been vaccinated.
Dengue vaccines are needed. Major questions related to the development of dengue vaccines arise due to the existence of multiple serotypes and the tendency for second infections to provoke serious, life-threatening illness, observed in dengue-endemic areas. Obstacles to vaccine development have included the need for a tetravalent product and the lack of an animal model for the disease, an in vitro correlate of protection, and a surrogate for vaccine efficacy. Several live tetravalent vaccines are currently under development. Developers and regulatory authorities have reached an informal consensus on the design and execution of clinical trials that could lead to licensure. One view of this consensus can be obtained by the WHO publication on this subject.

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