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Vaccination Against Dengue: Challenges and Current Developments

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Abstract

Dengue is a growing threat worldwide, and the development of a vaccine is a public health priority. The completion of the active phase of two pivotal efficacy studies conducted in Asia and Latin America by Sanofi Pasteur has constituted an important step. Several other approaches are under development, and whichever technology is used, vaccine developers face several challenges linked to the particular nature and etiology of dengue disease. We start our review by defining questions and potential issues linked to dengue pathology and presenting the main types of vaccine approaches that have explored these questions; some of these candidates are in a late stage of clinical development. In the second part of the review, we focus on the Sanofi Pasteur dengue vaccine candidate, describing the steps from research to phase III efficacy studies. Finally, we discuss what could be the next steps, before and after vaccine introduction, to ensure that the vaccine will provide the best benefit with an acceptable safety profile to the identified target populations.

INTRODUCTION: DENGUE DISEASE AND QUESTIONS TO BE ADDRESSED DURING VACCINE DEVELOPMENT

Dengue is a mosquito-borne disease caused by one of four closely related but antigenically distinct virus serotypes of the genus flavivirus. Dengue is a growing public health problem despite vector control efforts in many countries. An estimated 390 million dengue infections occur annually, and about 96 million people have clinically apparent dengue disease (1). As a result, the World Health Organization (WHO) considers the development of an effective dengue vaccine a high priority (**http://www.who.int/immunization/diseases/dengue/en/**).

About two-thirds of dengue infections result in asymptomatic cases, but some of these will lead to symptomatic "classical" dengue fever (DF, often presenting as a flu-like syndrome), which may progress in a few percent of cases into severe disease. In 1997, WHO classified symptomatic cases as (*a*) undifferentiated fever; (*b*) dengue fever (acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia); (*c*) dengue hemorrhagic fever (DHF; fever, hemorrhagic manifestations, thrombocytopenia, and plasma leakage); or (*d*) dengue shock syndrome (DSS; hypovolemic shock) (2). A new WHO classification was defined in 2009, dividing cases between engue "dengue without or with warning signs" and "severe dengue." The warning signs include abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed, lethargy, restlessness, liver enlargement >2 cm, and an increase in hematocrit concurrent with a rapid decrease in platelet count. These cases require strict observation and medical intervention. The criteria for severe dengue include (*a*) severe plasma leakage, possibly leading to shock (DSS), and fluid accumulation with respiratory distress; (*b*) severe hemorrhage, as evaluated by a clinician; and/or (*c*) severe organ impairment, e.g., liver findings including aspartate aminotransferase or alanine aminotransferase levels ≥1,000, impairment of the central nervous system manifesting as impaired consciousness, or impairment of the heart and other organs (3).

Notwithstanding the evolved classification of dengue disease, and the observation that high initial viremia levels are associated with severe cases (4), the severe forms of disease often appear when levels of viremia are already declining or when the virus is no longer detectable in the blood. Furthermore, viral infection usually does not result in direct cell or sustained organ damage (for review see 5). Therefore, it appears that severe dengue is mainly the consequence of an immunopathological reaction initiated early in the course of infection. This reaction involves innate responses (particularly in the case of primary infection), with the additional contribution of adaptive responses, particularly in the case of secondary infection, which is a risk factor for severity (see below; for review see 6, 7).

Briefly, innate responses represent the first line of defense against dengue virus and involve monocytes, macrophages, and myeloid dendritic cells (8–10), the latter being critical antigen-presenting cells in the initiation of primary responses. Dendritic cells in the skin are indeed among the primary cells infected after inoculation via mosquito bite, and they subsequently migrate to the draining lymph nodes (11), where they interact with T cells. Dengue virus can also infect and/or stimulate other types of antigen-presenting cells such as B cells (12) and plasmacytoid dendritic cells (13), the latter contributing significantly to the antiviral type I interferon (IFN) response. Dengue infection can also impact positively or negatively the activation of bystander uninfected cells (14). Natural killer (NK) cells are another early contributor to the control of viral infection (15), but upregulation of class I major histocompatibility complex molecules counteracts NK activity (16). Additional mechanisms linked to the activity of several nonstructural dengue proteins can counteract antiviral type I IFN responses (for review see 17). Several other players of the innate or vascular systems can also be modulated by dengue virus infection and expressed genes, such as

mast cells (18), endothelial cells (for review see 19), hepatic cells (20), and platelets (21); a defect or overactivation of some components of the complement system can also influence the progression of infection (22, 23). Overall, infection and/or indirect activation of one or several of these various cell types stimulate several pathways. In particular, their activation drives an antiviral response, including stimulation of some pattern-recognition receptors, type I IFNs, interferon-stimulated genes, and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) (24). Infection will also trigger the expression of a wide array of proinflammatory (or anti-inflammatory) cytokines and chemokines, such as interleukin (IL)-6, tumor necrosis factor α (TNFα), IL-1β, IL-10, IL-8, and chemokines CXCL9–11, CXCL1–3, and CCL2–5, whose levels and kinetics shape the outcome of the disease.

After initial replication at the sites of entry, virus appears in blood during the acute febrile phase, generally for 3–5 days, and may be recovered from serum and from peripheral blood mononuclear cells. Adaptive responses play an important role in secondary or subsequent dengue infection(s), which can be caused by a homologous or heterologous serotype. Protective immunity against reinfection by the same (homologous) serotype is considered to be life long, but reinfection by a heterologous serotype may lead to different outcomes, depending on the interval between the two infections. In the event of a secondary heterologous infection, pre-existing neutralizing antibodies (i.e., triggered by a previous infection) may reduce the initial infection of skin, lymph node, and circulating cells, and pre-existing cellular responses (T helper and cytotoxic T cells) may limit the expansion of infection by killing infected cells and secreting inflammatory cytokines (for review see 7, 25–28). Antibodies can also play an additional protective role through antibody-dependent cellular cytotoxicity (29). Both humoral and cellular arms can thus play a role in protection against dengue disease, but both arms have also been linked to immunopathology, acting detrimentally under certain conditions. In fact, beyond a certain "grace" or "honeymoon" period of time—from several months to three years—during which cross-reactive responses are cross-protective (30–35), responses induced by a primary infection may sensitize an individual to a more severe secondary infection caused by a different serotype. This phenomenon has been hypothesized to be linked in particular to non-neutralizing enhancing antibodies facilitating virus uptake through Fc receptors—a mechanism known as antibody-dependent enhancement (ADE)—and/or to a detrimental inflammatory or biased T cell response (for review see 6, 7). ADE may also bias cytokine responses through an "intrinsic ADE" mechanism, enhancing immunosuppressive IL-10 expression and counteracting antiviral responses (36).

Thus, both innate and adaptive mechanisms can contribute to eventually directing responses in a protective or nonprotective direction, and measuring these responses helps us to identify the most favorable type of immunity. The use of DNA arrays has allowed monitoring further the expression of the different cytokines, chemokines, and other gene products stimulated by dengue infection in vivo, whose differential expression may contribute positively or negatively to disease evolution (e.g., 37–39). Although innate responses shape subsequent adaptive ones, these latter responses may also contribute through memory cells to rapidly trigger some innate mechanisms and cytokines upon re-exposure to dengue virus.

By analogy with traffic lights, **Figure 1** synthesizes the immune parameters that may preferentially lead to protection or to severe disease. It is important to consider not only the nature of the immune mediators but also their level and kinetic appearance. Too much of a good thing is not necessarily beneficial, and some "detrimental" factors involved in innate or T cell responses may also play a positive role at the onset of disease or to terminate inflammatory reactions.

These observations need to be considered when defining and developing vaccination strategies. For instance, it is important to address the nature, kinetics, and level of early innate responses induced by vaccination, as well as to monitor triggered antibodies and cellular responses, in order

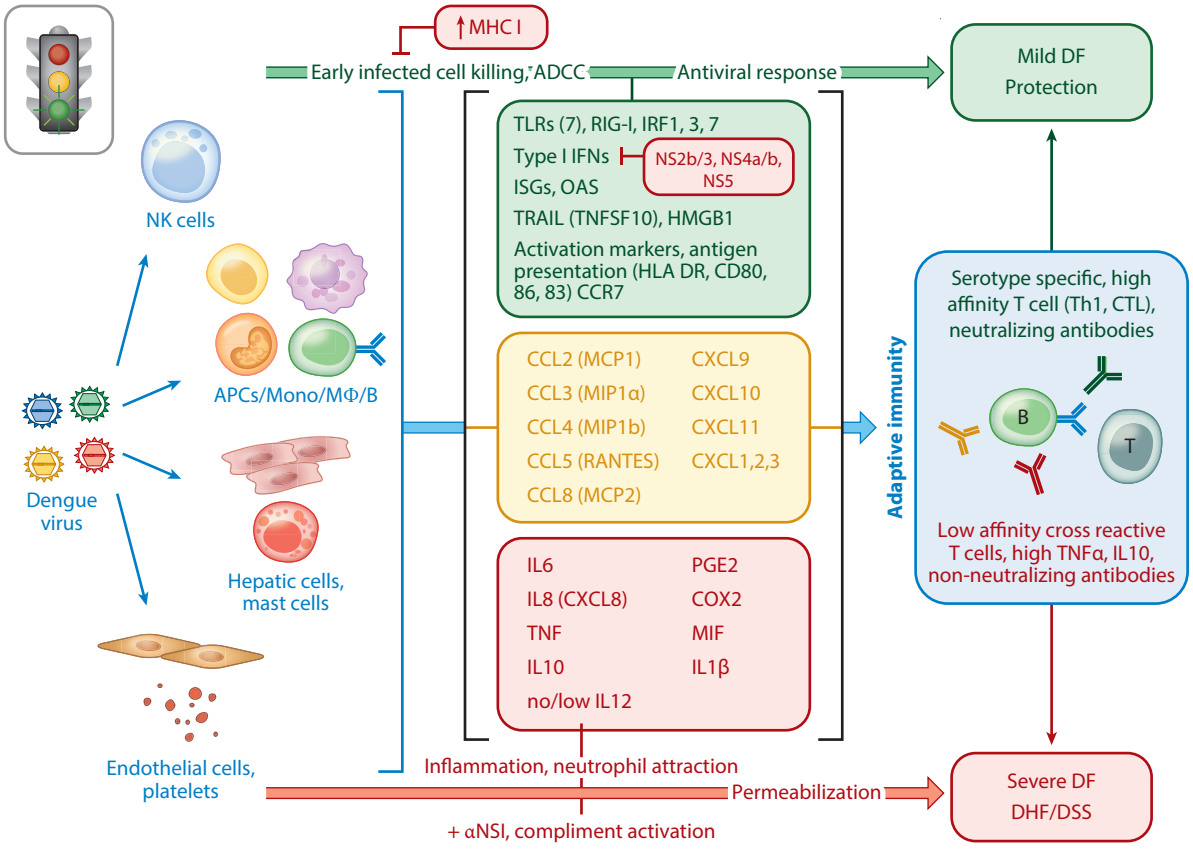

Figure 1

Innate and adaptive responses potentially involved in protection or severe dengue disease upon primary or secondary infection. By analogy with traffic lights, this figure presents a synthesis of the immune parameters that may preferentially lead to protection (the "green" factors, mainly representing a type I interferon antiviral response) or to severe disease (the "red" factors, mainly representing an excessive inflammatory or on the contrary an immunosuppressive response). Chemokines attracting neutrophils are also seen as deleterious. The "yellow" factors mainly correspond to chemokines linking innate and adaptive responses, and the nature, level, and kinetics of these factors may favor either a "green" or a "red" outcome. Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; APCs, antigen-presenting cells; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome.

to establish which profile(s) of response is (are) induced, and if such responses are most likely to be protective or enhancing. It should be noted that historically, a neutralization titer of ≥10 using a plaque reduction assay (PRNT50) was assumed to indicate seroconversion.

We describe in the following paragraphs the vaccine approaches currently under development in several laboratories and companies, focusing in the second part of this review on the most advanced candidate, i.e., the Sanofi Pasteur CYD dengue vaccine. With regard to the immune responses mentioned above, we also describe the investigations that have been carried out to characterize the immunogenicity, safety, and efficacy of this vaccine candidate.

VACCINE APPROACHES

Although no licensed vaccine is available yet, several promising candidates are under development (see **Figure 2**). The ideal dengue vaccine should provide life-long immunity against infection by

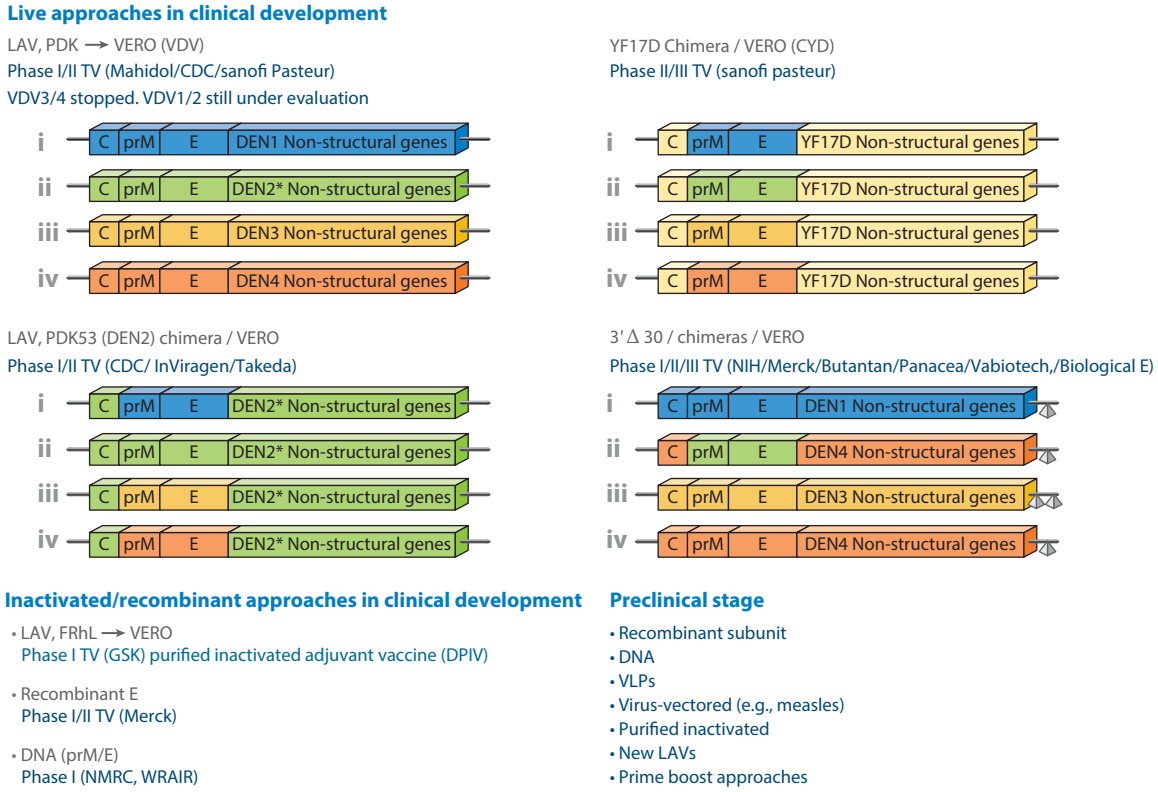

Figure 2

Vaccine approaches under development (40). The most advanced candidates are live attenuated vaccines (LAVs), which include live chimeras based on attenuated dengue virus or yellow fever (YF) backbones. Less advanced approaches include inactivated/recombinant adjuvanted vaccines and DNA vaccines. A large number of approaches (or combinations of technologies) are still at the preclinical level. Asterisk denotes 16681 PDK53 strain. Other abbreviations: CDC, Centers for Disease Control; DPIV, dengue purified inactivated vaccine; FRhL, fetal rhesus lung; GSK, Glaxo Smith Kline; NMRC, Naval Medical Research Center; NIH, National Institutes of Health; TV, tetravalent; VDV, vero dengue vaccine; VLP, Virus-like particles; WRAIR, Walter Reed Army Institute of Research.

any of the four serotypes and be free from any reactogenicity (see above). It should be suitable for use in children and provide immune responses that do not at any point in the vaccination process increase the risk of DHF from concomitant or subsequent exposure to wild-type virus.

As highlighted above, a vaccine should ideally induce both potent humoral (neutralizing antibodies) and cellular (Th1/cytotoxic T lymphocyte) protective immunity. Live attenuated vaccines (LAVs) should be optimal in this respect. Attenuated strains must be able to replicate sufficiently well in vivo to provoke an immune response (ideally against all four serotypes at the same time) but with minimal systemic replication to avoid the induction of dengue-associated symptoms of fever, headache, and arthralgia. The strains should not cause high viremia, given observations that severe disease can be generally associated with higher levels of viremia (4). As a potential benchmark, low levels of viremia resulting from the 17D yellow fever vaccine virus (YFV17D) may be acceptable based on decades of extensive use of this vaccine. LAV strains also need to be genetically stable for critical attenuation mutations because any reversion, either during vaccine batch manufacture or following administration, may adversely affect safety. Moreover, the strains must be incapable of transmission by mosquitoes, since this may facilitate evolutionary change toward virulence. Such

transmission is unlikely if viremia is low, but mutations restricting replications in the mosquito host are also desirable.

The best-studied first-generation "classical" LAV strains were developed at Mahidol University in Bangkok and at the Walter Reed Army Institute of Research and were derived by the usual empirical method of multiple passages in cell culture, principally primary canine kidney cells and fetal rhesus lung cells (41, 42). Derivatives of the Mahidol strains have also been adapted by Sanofi Pasteur for growth in Vero cells (Vero dengue vaccine; VDV) (43). However, the first evaluation of the serotype 3 VDV (VDV3), used at a low dose, resulted in unacceptable reactogenicity (43), which stopped the development of this approach for a tetravalent vaccine. Sanofi Pasteur then focused its development on the ChimeriVax technology using YFV17D as a backbone (see below and 44).

A different approach was adopted by the National Institutes of Health (NIH) Laboratory of Infectious Diseases, based on reverse genetics. Here, attenuation was achieved by creating a 30-base deletion (Δ30) in the 3′ untranslated region (UTR) of the genomes of the four serotypes of dengue virus (45). This strategy was successful for serotypes 1 and 4 but did not generate suitable candidates for serotypes 2 and 3. An additional deletion was performed for serotype 3 (double deletion Δ30/31), and the serotype 2 candidate was generated by constructing a chimera based on the attenuated serotype 4 strain as backbone, carrying the premembrane (prM) and envelope (E) genes of serotype 2 (45). Different versions of individual monovalent vaccines were tested in humans, followed by the definition of an optimal tetravalent formulation in a phase I trial. Development then moved to phase II with formulations TV003 and TV005. The former formulation contained 3 log plaque forming unit (PFU) of each serotype, and the latter contained a higher dose (4 log PFU) of serotype 2. Recent results showed satisfactory reactogenicity and immunogenicity for the TV005 formulation after one dose; mild rash was common following the first vaccination, occurring in ∼60% of volunteers (46). The NIH technology has been nonexclusively licensed to different local manufacturers in countries where dengue is endemic, such as Instituto Butantan in Brazil, and was also licensed to Merck & Co. (see **Figure 2**). Butantan announced in 2015 the intention to launch of a large-scale phase III efficacy trial in a single country (Brazil), in volunteers aged 2 to 59 years who will receive one dose of tetravalent vaccine (NCT02406729).

Also in the LAV category, the Centers for Disease Control and Prevention (CDC) Division of Vector-Borne Diseases (United States) developed an approach using the attenuated Mahidol serotype 2 strain, passaged in primary dog kidney cells (strain 16681, PDK53). This approach was further developed by Inviragen, Inc. (United States), and is now supported by Takeda Pharmaceutical Co., Ltd. (Japan) (47). Three key attenuation mutations are present respectively in the 5′ UTR and in two nonstructural (NS) genes of DEN2 PDK53; this strain can thus constitute a backbone for building serotypes 1, 3, and 4 chimeras after the corresponding structural prM and E genes of these serotypes are exchanged with those of PDK53 (48, see **Figure 2**). As for the NIH approach, satisfactory immunogenicity and safety have been demonstrated in both preclinical models and phase I clinical trials, with dominant responses induced against serotype 2 (48). Development is in clinical phase IIa studies.

Both the NIH and CDC/Takeda approaches induce cellular responses against nonstructural dengue antigens (DEN2 for Takeda; DEN1, 3, and 4 for NIH), in addition to those triggered against structural antigens (prM/E). The potential role of such responses in protection will be addressed in coming efficacy trials.

The difficulty of designing strains of a sufficient level of attenuation that are immunogenic and noninterfering when administered as a tetravalent mixture has led other groups to concentrate on inactivated vaccine approaches. Although less likely to induce a cellular response comparable to natural infection (involving both CD4 and CD8), killed and adjuvanted vaccines offer in principle

a more reliable way of achieving a balanced immune response against all four serotypes at the same time, thus potentially lowering the risk of sensitization to severe disease (see above).

Figure 2 shows a nonexhaustive list of such nonlive approaches. The most advanced from a clinical standpoint are those developed by GlaxoSmithKline (inactivated adjuvanted vaccines) and Merck (recombinant 80% E) (49–50).

THE SANOFI PASTEUR VACCINE APPROACH

At the end of the 1990s, scientists from Acambis (United States; acquired by Sanofi Pasteur in 2008) applied to dengue a concept and technology that had initially come from NIH and subsequently developed at Washington University St. Louis (United States), using YFV17D as a backbone. This technology, ChimeriVax (52), utilizes the construction of chimeras by exchanging the structural prM/E genes of YFV17D with the corresponding genes of each dengue serotype (see **Figure 2**). The parental strains of the four chimeric dengue vaccines (named CYD-1 to -4) are as follows: Thai strain PUO-359/TVP-1140 for CYD-1, Thai strain PUO-218 for CYD-2, Thai strain PaH881/88 for CYD-3, and Indonesian strain 1228 (TVP-980) for CYD-4.

The objective of this strategy was to retain the well-characterized attenuation phenotype of the YFV17D backbone and incorporate dengue antigenicity. The initial preclinical and clinical development of these chimeras are presented below, followed by the results of two recent pivotal phase III efficacy studies.

PRECLINICAL DEVELOPMENT

The first steps in CYD dengue vaccine development were in vitro and in vivo preclinical evaluation, nonclinical safety evaluation, and environmental and theoretical risk assessment. The CYD dengue vaccine is composed of four chimeric LAVs, and it was thus important to document in particular several aspects potentially linked to their live and recombinant nature. This was done in accordance with WHO guidelines (53), and a template has also been recently proposed by the Brighton group regarding YFV17D-based vaccines (54). The following conclusions were noted (for review see 44, 52).

The four CYD dengue viruses present satisfactory genetic stability from early passages to bulk stages. Plaque size phenotypes are stable at all production steps, and low and stable neurovirulence was observed in a suckling mouse model (55, 56).

Regarding post-translational modifications, high mannose and complex/hybrid glycosylation are observed at both sites (N67 and N153) for all four CYD dengue virus serotypes. These observations are in agreement with their ability to interact with the DC-SIGN (CD209) coreceptor and dendritic cells (57).

Regarding in vitro infectivity and immunogenicity, CYD-1–4 show similar growth kinetics to those of their parent viruses (wild-type DEN and YFV17D) in human monocyte-derived dendritic cells (mDCs) (58). Such mDC infection induces maturation and a controlled innate response, accompanied by limited inflammatory cytokine production and consistent expression of antiviral type I IFN and chemokines linking innate and adaptive responses (59, 60). The four serotypes also grow to significantly lower titers than YFV17D in human hepatic cell lines THLE-3 and HepG2 (58).

Regarding in vivo immunogenicity, the tetravalent CYD-1–4 vaccine is immunogenic in monkeys, induces limited viremia, and protects against challenge with wild-type DEN (viremia) (61, 62). Monovalent CYD-2 also induces some protection and more rapid clearance after a virulent DEN2 challenge (63). In the same animal model, interference at the replication or immunological level between serotypes can be mitigated by relative serotype dose and/or schedule adjustment (64).

Evaluation of nonclinical safety included a repeat dose with local tolerance, a biodistribution and shedding analysis as well as neurovirulence and DART (developmental and reproductive toxicology) studies. Distribution of CYD dengue viruses was limited at low levels to the injection site, the lymphoid tissues, and/or the liver, and was transient. There was no evidence of neurotropism. The pivotal developmental and reproductive toxicity studies in rabbits and mice given CYD dengue vaccine IV at the human dose showed no adverse effects on the mating performance and fertility of the vaccinated rabbits and showed no teratogenic potential and no effect on pre- and postnatal development in rabbits and mice (65).

Several specific theoretical risks have been assessed. We observed a lack of transmission by laboratory and field *Aedes aegypti* and *A. albopictus* mosquito vectors was observed, as well as a lack of transmission by ticks. Along with the low level of viremia in the host, this observation implies little or no risk of dissemination of vaccine viruses in the environment (66, 67). We also addressed a potential reversion to virulence and showed that it is not possible to create a wild-type virulent yellow fever virus in vaccinees because (*a*) the YFV17D envelope genes are missing and (*b*) numerous reversions to wild type of attenuating residues within the seven nonstructural genes and the core protein gene are required. Similarly, natural recombination is highly unlikely, as shown by absence of recombinants in forced in vitro systems (68). Moreover, we generated data with artificial recombinants, which provided strong evidence that, should they ever emerge, they would not cause disease or spread in the environment (69, 70). Regarding viscerotropism, we observed that CYD dengue viruses are less hepatotropic and less neurotropic than YFV17D (55, 71). These theoretical risks are also carefully addressed in clinical trials and will be included in postmarketing surveillance programs.

We also addressed the risk of sensitization/ADE since the early stages of development (72) and throughout the successive and ongoing clinical trials (short-term and long-term follow-up). As for viscerotropism, careful evaluation for sensitization potentially linked to ADE will be part of postmarketing surveillance. In vitro ADE assays so far have not identified serotype-specific differences in enhancing antibodies that could be linked to different serotype-specific efficacy in phase III trials (73). In the event that long-term observation studies demonstrate waning efficacy, booster vaccinations will be considered.

EARLY CLINICAL DEVELOPMENT

All clinical development for the CYD vaccine complies with international guidelines for new vaccines (i.e., the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, the US Food and Drug Administration, and the European Medicines Agency), as well as the WHO Technical Report Series No. 932 guidelines for the production and quality control of live candidate tetravalent dengue virus vaccines (53). Because flavivirus immunological background rates vary between regions, clinical trials have been conducted on several continents in children, adolescents, and adults with diverse flavivirus infection and vaccination histories. Overall, 29,000 subjects aged 9 months to 60 years of age have received at least one dose of the CYD dengue vaccine in a total safety database of more than 41,000 subjects. Initial clinical evaluation addressed safety [including serious adverse events and adverse events of special interest (e.g., viscero- or neurotropic diseases, allergic reaction, severe dengue)], reactogenicity, vaccine viremia, and immunogenicity (antibodies and T cells). **Supplemental Figure 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**) presents the main investigations linked to clinical trials in humans, with key steps, topics, and related questions.

Phase I and II trials in subjects ranging from toddlers to adults indicated that CYD-1–4 induce neutralizing responses (in a PRNT50 assay; 74, 75) and are nonreactogenic. Flavivirus/dengue preimmunity is linked to higher and broader neutralizing responses (76–82).

Regarding cell-mediated immunity, tetravalent CYD dengue vaccine induces antidengue serotype-specific CD4 (Th1) and anti-YFV17D NS3-specific CD8 responses. Dengue preimmunity or booster immunization broadens cell-mediated immunity. Immunization in dengue-positive individuals recalls anti-DEN NS3 responses (83–85).

These studies have identified no safety issues. Long-term safety assessment capturing hospitalized cases of dengue through passive surveillance is ongoing in phase IIb and III efficacy trials (see below; 86–88). **Figure 3** presents a synthesis of the major immunogenicity results obtained in early preclinical and clinical development.

RECENT CLINICAL DEVELOPMENT

Following these satisfactory initial preclinical and clinical evaluations, three efficacy trials were launched, and results from their active phase have been released in the past two years. These included a proof-of-concept phase IIb efficacy study in Thailand and two pivotal phase III efficacy trials in Asia and Latin America. The first priority has been to develop the vaccine in regions where dengue is endemic (Asia-Pacific countries, Latin America, and the Caribbean) to address the unmet medical need there.

First-Proof-of-Concept Efficacy Study

CYD23, a first-proof-of-concept monocentric phase IIb clinical efficacy study (NCT00842530), was conducted in 4,002 healthy Thai schoolchildren aged 4–11 years, randomly assigned (2:1) to receive three injections of dengue vaccine or control (rabies vaccine or placebo) at months 0, 6, and 12 (86). Participants were actively followed until month 25 (active phase). All acute febrile illnesses were tested for dengue, and viremia was confirmed by serotype-specific reverse transcription PCR (RT-PCR) and nonstructural protein 1 ELISA, as in the subsequent phase III trials (see below). The primary objective was to assess protective efficacy against virologically confirmed, symptomatic dengue, irrespective of severity or serotype, occurring one month or longer after the third injection (per-protocol analysis). Incidence of disease was high in the trial, and the larger-than-expected number of cases was beneficial to address endpoints that would otherwise not have been possible to consider, including serotype-specific efficacy. A similar situation was encountered in the two subsequent phase III trials (see below). In CYD23, efficacy according to the primary endpoint was 30.2% (95% CI, 13.4–56.6) and differed by serotype. In particular, no efficacy was observed in the trial against serotype 2, despite PRNT50 levels similar to those induced against the other serotypes. The dengue vaccine was well tolerated, with no safety signals after two years of active follow-up after the first dose. These data showed for the first time that a vaccine against dengue was feasible, and they raised several important issues—in particular, the link between neutralizing levels and protection, and the importance of varying serotype-specific efficacy. The subsequent hospital phase surveillance for the phase IIb study (CYD57) continues until 2016.

Pivotal Phase III Efficacy Studies and Long-Term Follow-Up of Hospitalized Cases of Dengue

Following CYD23, the active phases of two pivotal phase III efficacy studies were carried out. CYD14 (NCT01373281) enrolled ~10,000 children aged 2–14 years in Asia (87), and CYD15

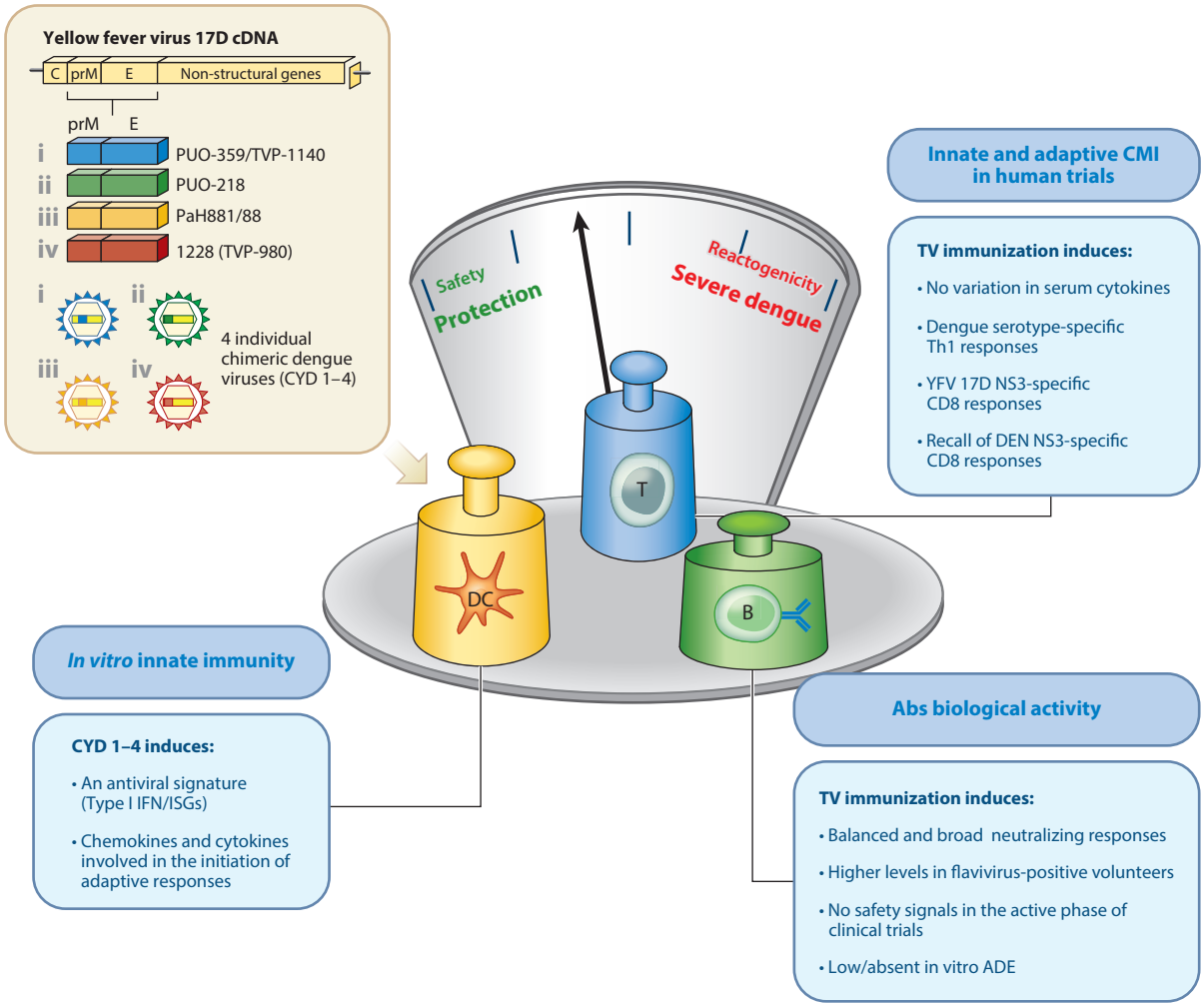

Figure 3

In vitro and in vivo innate and adaptive responses triggered after infection/vaccination with CYD dengue viruses. Three main players shape the immune response induced by infection or vaccination: the antigen-presenting cells such as the dendritic cells, the T cells (CD4 and CD8), and the B cells. According to the quality and level ("weight") of the responses induced by these cells, overall response ("immune balance") may point toward safety/protection or reactogenicity/severity. Preclinical and early clinical findings established the satisfactory safety/reactogenicity and immunogenicity of the CYD dengue vaccine, in vitro and in vivo, at the innate, T cell, and B cell levels. Abbreviations: ADE, antibody-dependent enhancement; CMI, cell-mediated immunity.

(NCT01374516) enrolled ~20,000 children and adolescents aged 9–16 years in Central and South America (88). Each trial included five countries where dengue is endemic, and, like CYD23, examined the efficacy of a three-dose schedule (0, 6, and 12 months) of CYD tetravalent dengue vaccine (CYD-TDV) to reduce symptomatic, virologically confirmed dengue during a period of 12 months starting 28 days after the third dose (per-protocol analysis). In both CYD14 and CYD15, the primary endpoint was for the lower bound of the 95% confidence interval (CI) of vaccine efficacy to be >25%. The design and main results of the active phase of these studies are

presented in **Supplemental Figure 2** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**).

The active phase of the two trials has now been completed, providing efficacy and safety results from the first 25 months following the initial vaccination. In the Asian trial, efficacy against virologically confirmed dengue was 56.5% (95% CI, 43.8–66.4) in the per-protocol analysis, irrespective of disease severity against any serotype. Similarly, in the Latin American trial, efficacy against virologically confirmed dengue was 60.8% (95% CI, 52.0–68.0) during the 25-month active phase. Thus, both trials met their primary endpoint. Secondary analyses in the Asian study showed that all four dengue serotypes contributed to the overall efficacy during the active phase, although efficacy against serotype 2 was measurable but inconclusive (lower bound of the 95% CI < 0); efficacy against all four serotypes was conclusive in the Latin American trial. Both trials also showed during the active phase higher efficacy against severe disease and hospitalized cases of dengue. An intent-to-treat analysis demonstrated, in Asia, 54.8% overall efficacy against dengue disease versus 67.2% efficacy against hospitalization and 80.0% efficacy against DHF, and in Latin America, 64.7% overall efficacy against dengue disease versus 80.3% efficacy against hospitalization and 95.0% efficacy against DHF.

A meta-analysis of efficacy was also performed for the active phase of CYD14 and CYD15, and further analyses of hospital phase surveillance data (see below) prompted a focus for the pooled analysis on an age cut-point of ≥9 years, which was also the lower age of participants enrolled in CYD15 (89). Vaccine efficacy against VCD of any severity due to all serotypes in the pooled analyses for participants aged ≥9 years in the active phase was 65.6% (95% CI 60.7; 69.9). Pooled vaccine efficacy for seronegative individuals aged ≥9 years was 52.5% (95% CI 5.9; 76.1). Vaccine efficacy was also confirmed in individuals who were seropositive at baseline, 82% (95% CI 65.4; 86.5).

Both phase III trials identified prior exposure to dengue as an important covariate for efficacy, finding higher protection in participants who had been previously exposed to dengue (seropositive by PRNT50) than in seronegative participants. Efficacy also increased with age in the CYD14 trial (87), the increase in age reflecting accumulative exposure to dengue and therefore serving as a surrogate of seropositivity. In both trials, during the 25-month active phase, the safety profile for the vaccine was similar to that for placebo, with no marked differences in rates of adverse events. The safety findings are in agreement with the published results from prior clinical trials (see above).

Beyond the active phase, the clinical development program for the CYD-TDV candidate vaccine includes a four-year long-term follow-up (LTFU) phase, i.e., the Hospital Phase, starting 13 months after the third vaccine administration and ending five years after completion of the vaccination schedule, to assess safety in line with the WHO guidelines. Hospitalization for acute fever is recorded during study contacts, and by self-reporting and surveillance of the hospital network.

During the first year of the LFTU in the multicentric Asian trial CYD14, there was a trend for a higher risk of hospitalized symptomatic VCD in the vaccine group despite the low number of cases observed (89). Preplanned analyses showed that the risk was higher in younger children, particularly in the youngest age group analyzed, 2–5 years. This shift in relative risk (RR) for hospitalized VCD directed us to address it separately in populations younger and older than nine years. No issue was seen in the ≥9 years age group in CYD14 and CYD57 (follow-up of the CYD23 phase IIb trial) (86), and similarly no issue was seen in CYD15 in the preplanned ≥9 years analysis. Pooled RRs during the first year of LTFU of CYD14, CYD15, and CYD57 were 0.84 (95% CI 0.56; 1.24), 1.58 (95% CI 0.83; 3.02), and 0.50 (95% CI 0.29; 0.86) for all participants, those aged <9 years, and those aged ≥9 years, respectively. Importantly, the clinical profile of severe

hospitalized symptomatic virologically confirmed dengue cases during LTFU is not different from that observed during the active surveillance phase. Cumulative RRs (active phase and first year of LTFU) were as follows: CYD14, 0.46 (95% CI 0.32; 0.65), CYD15, 0.28 (95% CI 0.18; 0.44), and CYD23/57, 0.66 (95% CI 0.43; 1.02). In totality, these analyses suggested that the optimal age for intervention is from nine years, given the observed favorable clinical profile with higher efficacy for preventing VCD and an acceptable post-vaccination safety profile for individuals aged ≥9 years.

QUESTIONS RAISED BY CLINICAL TRIALS

Overall, both phase IIb and phase III results identified important determinants of efficacy with the CYD vaccine technology, in particular the varying serotype-specific efficacy, the importance of dengue baseline preimmunity, and the potential importance of age as a surrogate of prior exposure. In addition, age could reflect some immaturity at both immunological and physiological levels (90, 91). These aspects have been and are still being addressed through investigations by Sanofi Pasteur and/or through external collaborations (92). The efficacy trials showed higher protection against serotypes 3 and 4 than against serotypes 1 and 2, while similar neutralization geometric mean titre values (using the Vero cell–based PRNT50 assay) (74, 75) were observed for all four serotypes. Post-study investigations to understand this result included a broad array of analytical and experimental methods in four areas: host and immunity, virus, vaccine, and vector (92, 64). To date, results suggest that despite the presence of key epitopes on the vaccine viruses, qualitative differences exist in responses against the different serotypes in naïve volunteers and responses are qualitatively and quantitatively different in dengue-preimmune volunteers (64), although it is necessary to further confirm the results obtained in a larger number of sera. A human challenge model, such as the one developed by the Walter Reed Institute, will play an important role in addressing some of these aspects (93).

Ongoing long-term clinical follow-up and investigations will continue to bring up additional critical elements, in particular regarding long-term protection and safety in the different age groups. This will be informative in particular on the evolution of the imbalance in VCD observed in the first year of the hospital phase in vaccinees aged <9 years (89); in this regard, several interrelated biological hypotheses involving waning immunity, age/serostatus, and temporal clustering of infection in vaccinees are under investigation (B. Guy, N. Jackson, manuscript in preparation). Assuming successful licensure, the risk-management plan established up front will be important to support the benefit–risk profile of the vaccine.

INDUSTRIALIZATION

In parallel with preclinical and clinical investigations, it was important to develop a robust industrial process in order to supply the vaccine used in phase III trials and to anticipate the needs when the vaccine is licensed. The production process, summarized elsewhere (44), was set up to ensure a reliable and consistent supply of virus and cells at the industrial level. In 2006, the process was transferred to the Industrial Operations Division of Sanofi Pasteur, and a production facility was dedicated in Marcy l'Etoile, France, using industrial-scale biogenerators to produce the cells and virus.

In 2008, four years before the first phase IIb clinical trials, Sanofi Pasteur decided to create a new vaccine production site in Neuville sur Saône, France, to anticipate future needs and to be able to provide this new vaccine as soon as possible after licensure. The Neuville production facility scales up the Marcy l'Etoile facility and can produce virus (drug substance) to provide up

to 100 million doses per year. The consistency lots have already been produced and will ensure the availability of the vaccine at an industrial scale in the coming years.

NEXT STEPS LINKED TO VACCINE INTRODUCTION

Catch-up program: Mass vaccination campaign at the beginning of a vaccination program targeting people not eligible for routine vaccination

The development and production of a safe and efficacious vaccine are the first steps to ensuring the protection of the vaccine-targeted populations at risk from dengue (i.e., age ≥9 years). However, other challenges—epidemiological, economic, regulatory, medical, and logistical—must also be met to ensure the successful introduction of the vaccine into routine schedules and catch-up programs, in order to significantly reduce the burden of disease in a given area where it is endemic (44).

One of the main challenges is determining the true burden of dengue disease. Estimates of disease incidence and burden that rely solely on the number of reported cases will inevitably underestimate the magnitude of the problem. Improved surveillance systems will be needed to quantify the medical value of the dengue vaccination programs in studies of effectiveness and vaccination coverage.

The epidemiology of dengue varies considerably, both geographically and temporally. Such epidemiological specificities may require vaccination programs to be tailored regionally or nationally. Existing immunization programs represent another national specificity that must be accounted for, as the introduction of dengue immunization must not detract from existing programs.

Dengue immunization should also be considered as part of a wider, integrated strategy with community involvement, surveillance, case management, and vector and outbreak control. Governments will need to anticipate budget needs for routine dengue vaccination, catch-up programs, consumables, infrastructure, training, and surveillance. Alternative funding mechanisms will be needed to finance vaccination programs in some countries where dengue is endemic. Mathematical modeling is an important tool for assessing the parameters described above, specific for a given country and epidemiological setting, and predicting the impact of vaccination on the burden of disease over time.

The initial introduction of dengue vaccination will be accompanied by long-term phase IV studies, which should be planned in collaboration with national authorities. These will serve to demonstrate the medical value (including effectiveness and safety) of dengue vaccination and constitute requirements for risk-management plans.

CONCLUSIONS

The end of the active phase of the first two phase III pivotal efficacy field studies closed more than a decade of development of the CYD dengue vaccine, during which its developers had to tackle complexity, pursue innovation, and manage risk. Safety, tolerability, and immunogenicity induced by CYD-TDV have been extensively characterized in preclinical and clinical studies. Results of the phase III efficacy trials conducted in Asia and Latin America have consistently demonstrated that the vaccine is safe and effective against all four dengue serotypes during the 25-month active phase. High efficacy against the severe forms of disease and a reduction in hospitalized dengue cases were also observed during the 25-month active phase. Importantly, serotype distribution and dengue serostatus prior to vaccination appear to impact vaccine efficacy, and recent data obtained after the first year of follow-up in the hospital phase further allowed us to define the vaccine target population: from 9 to 60 years of age. If this population is vaccinated, we conclude that the CYD vaccine has the potential to significantly reduce the burden of disease in countries debilitated by dengue. A significant number of post hoc analyses and experimental

investigations remain necessary to address scientific, clinical, and immunological questions, and, as stated above, to prepare for the introduction and use of the Sanofi Pasteur dengue vaccine. In vaccine implementation, and the development of modeling tools and the conduct of effectiveness studies will support the achievement of this goal. The risk-management plan we defined up front will also support a safe and efficient large-scale vaccine implementation.

As discussed, dengue is a complex disease, and both short-term and long-term safety and efficacy will have to be considered further to assess the overall benefit of the vaccine for human health. These will be addressed by ongoing long-term follow-up and future post-licensure studies. These questions and investigations may be relevant not only to CYD-TDV but also to other vaccine approaches in development (40, 50).

In conclusion, the development of a dengue vaccine requires a long-standing and continuous effort from private and public organizations to eventually bring a solution to the still growing and worldwide problem represented by dengue (1, 6). Several promising approaches are now in the late stages of clinical development, and it is reasonable to expect that one or more vaccines will be available in the coming years, which will bring a significant benefit to human health.

DISCLOSURE STATEMENT

All authors are employees of Sanofi Pasteur, and B.G. is a coauthor on several patents related to the Sanofi Pasteur dengue vaccine candidate.

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