The Necessity and Quandaries of Dengue Vaccine Development

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(See the article by Durbin et al, on pages 327–334).

Dengue is an emerging and reemerging arboviral disease of great global public health importance. Increased transmission and disease outbreaks are being driven by population growth, urbanization, international travel, and unchecked vector populations [1]. Southeast Asia, Central and South America, and parts of the Caribbean experience endemic and hyperendemic dengue virus (DENV) transmission while indigenous transmission is being increasingly recognized in areas of Africa, the Middle East and South Asia [2–5]. Reports indicate that southern US border-states and Hawaii can support episodic DENV transmission [6–12]. Dengue poses a risk to traveler and military populations, especially those originating from non-dengue endemic regions [13–18].

Millions of DENV infections, hundreds of thousands of hospitalizations, and tens of thousands of deaths related to dengue occur annually [19]. There is no specific, licensed anti-DENV therapeutic or preventative vaccine. The financial, social and individual cost of dengue is significant, underestimated, and underappreciated [20–25]. The strategic administration of a safe and efficacious dengue vaccine, in coordination with efforts to educate about personal protective measures and sustained vector control, is the best hope to reduce the global dengue burden.

There are numerous dengue vaccine candidates in clinical development. Early efforts to develop a dengue vaccine date back more than 70 years, with attempts to prevent virus transmission using infectious human plasma treated with ox bile or virus grown in live mosquitoes and inactivated with formalin [26]. Schelsinger and Sabin undertook the first attempts to immunize using mouse-passaged live-attenuated DENV-1 and -2 viruses [27–29]. Halstead and colleagues discovered DENVs were attenuated following passage in primary dog kidney (PDK) cell culture [30]. Mahidol University and Sanofi Pasteur attempted to codevelop live attenuated virus dengue vaccine candidates using PDK cell passage; the Walter Reed Army Institute of Research and GlaxoSmithKline Biologicals also used PDK passage to attenuate vaccine virus strain candidates [31–40]. The US National Institutes of Allergy and Infectious Diseases attenuate DENV strains by targeted mutagenesis; the resulting attenuated DENV strains may constitute stand-alone vaccine candidates or serve as chimeric backbones [41–45]. The US Naval Medical Research Center has completed a phase 1 trial testing a DENV-1 pre-Membrane/Envelope DNA vaccine; explorations of different vector and/or adjuvant combinations continue [46–50]. Hawaii Biotech/Merck & Company is completing a phase 1 trial testing a DENV-1 recombinant Envelope protein candidate [51–53]. Sanofi Pasteur is in advanced clinical development (phase 3) of a -chimeric-Yellow fever-dengue (CYD) vaccine candidate using a construct created at the St. Louis University Health Sciences Center and Acambis Inc. [54–59]. The CYD candidate is the first candidate to enter clinical endpoint trials.

Efforts to develop a dengue vaccine have been plagued by numerous challenges and quandaries, some established and others hypothetical. The most obvious quandary for the dengue vaccinologist is the existence of 4 DENV types, each capable of causing severe dengue and death. The global epidemiology of dengue and cocirculation of multiple DENV types within tight geographic areas mandates the need for a vaccine capable of protecting against disease caused by any DENV type, a tetravalent vaccine (ie, containing

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DENV-1-4 antigens) [2, 60]. The implication of this requirement extends well beyond the need to produce 4 DENV monovalent vaccines and then successfully combine into a single tetravalent formulation. Human and non-human primate studies have demonstrated the existence of DENV type-specific immune dominance/interference when administering tetravalent vaccine candidates, with the result being an imbalanced immune response, a very undesirable outcome (discussed below) [61, 62]. Whether this is a phenomenon and challenge only for live virus replicating vaccines or all tetravalent dengue vaccines is unknown.

The absence of a validated animal model of dengue disease is also a challenge to the vaccine development effort. Research in this area continues but at this time vaccine developers are without a reproducible and relevant disease model to initially test vaccine candidates, down-select promising formulations, and understand, early on, the potential for clinical benefit [63–67]. As a result, numerous, small-scale phase 1 and 2 human trials are required to advance candidates.

In addition to the absence of an animal model, there is no validated human challenge model. Timelines for developing and down-selecting malaria vaccine candidates have benefitted greatly from the *Plasmodium falciparum* human challenge model developed at the WRAIR [68]. Experimental human infection with DENV has been reproduced in hundreds over the last century without untoward effects [26–28, 69–73]. During the past decade the WRAIR has attempted to validate minimally attenuated DENV-1–4 vaccine candidates as human challenge strains with limited success [74]. The requirement for cGMP manufacture and complex regulatory and human subjects' protection requirements make this pursuit very resource intensive. It is unclear how a dengue human challenge model would need to perform (ie, reproduce dengue fever versus reproduce viremia with or without symptoms) to support dengue vaccine development plans.

The incomplete understanding of what "immune profile" will lead to a protective or pathogenic response following a DENV infection poses another challenge to developers. Although anti-DENV neutralizing antibodies are likely required for protection from dengue disease, it is well established certain antibody characteristics (ie, non-neutralizing, cross-reactive, low affinity) may contribute to a poor clinical outcome [75–80]. Additionally, cellular immunity plays a role in both protective and pathogenic outcomes following exposure [81–85]. Dengue vaccine candidates would, ideally, induce immune responses corresponding to protective, rather than pathogenic, profiles. The spectrum of immune profiles induced by dengue vaccine candidates using different approaches (eg, live virus, chimeras, DNA) is unclear, but vaccine developers are devoting resources to broadly characterize responses [86–92].

There is no established dengue immune correlate of protection. An immune correlate would support (1) understanding how vaccine immunogenicity relates to protection from disease; (2) generalizing efficacy across different populations; (3) facilitating bridging between clinical studies; and (4) defining the relevant parameter to establish vaccine potency tests [93]. Without a validated human challenge model, attempts to define an immune correlate will need to be made in the context of clinical endpoint trials.

The dengue vaccine field is also challenged by the biologic assays currently available to measure immunogenicity. The measurement of neutralizing antibody is the most relevant endpoint to the vaccine development effort from a scientific and regulatory perspective; neutralizing antibody is believed to be protective and is consistently measurable. Variations on the plaque reduction neutralization test (PRNT) or microneutralization assay platforms are currently used to measure neutralizing antibody [94, 95]. Unfortunately, assay results can be variable and, in the face of multiple antigen exposure (ie, secondary infection or vaccination with tetravalent dengue vaccines), difficult to interpret whether homotypic, high-quality antibody (ie, neutralizing and protective) or cross-reactive antibody is being measured [96]. Furthermore, each developer utilizes methods and reagents specific for their vaccine candidate, making inter-developer immunogenicity comparisons nearly impossible [97]. The Pediatric Dengue Vaccine Initiative and World Health Organization have attempted to facilitate standardization and harmonization of the PRNT across laboratories [95]. Dengue vaccine efficacy trials and associations between neutralizing antibody measurements and various clinical outcomes will improve our understanding of immunogenicity endpoints.

A major theoretical concern is that poorly immunizing vaccines (ie, low antibody titer, no induction of T or B cell memory), imbalanced responses (ie, variable antibody responses to each DENV type), or waning immunity (ie, decline in antibody titer over time) may increase vaccine recipient risk of an immunopathologic response (ie, enhanced disease) following subsequent natural infection or re-immunization [60]. Limited, long-term studies of dengue vaccine recipients residing in dengue-endemic areas and being exposed to natural infections have not revealed an increased risk of severe disease [98]. In this issue of *the Journal,* Durbin and colleagues [99] describe the results of experiments exposing recipients of attenuated monovalent dengue vaccines to heterotypic monovalent vaccines 0.6–7.4 years later. There were no overt safety signals observed compared with control (dengue vaccination in naïve volunteers), and neutralizing antibody profiles in the heterotypic group were broad, qualitatively mimicking what

is seen in natural secondary dengue virus infections. There are limitations in equating these experiments with natural secondary DENV infections (ie, higher risk for severe disease) or extrapolating results to potential dengue vaccine recipients who are primed with a natural DENV or another flavivirus (ie, Yellow fever virus or Japanese encephalitis virus), but the investigators provide an important step in assessing the safety of vaccinating DENV immune subjects. Early dengue vaccine efficacy trials will require long-term subject follow-up or phase 4 studies to better define the risks of immunizing populations in dengue endemic areas and areas where other flaviviruses circulate [60].

The global burden of dengue is significant and the world needs a dengue vaccine. The recent infusion of financial resources into dengue research greatly expanded what was once a narrow field of dengue scientists and funding entities. There are many dengue vaccine development initiatives underway, with numerous candidates in preclinical and clinical development. Data from the first dengue vaccine efficacy trial are greatly anticipated. Challenges to the vaccine development effort exist and there is much to learn. The worsening financial and societal burden of dengue calls for increased funding to facilitate the study, and improved understanding of dengue epidemiology, immunology, and the development and advancement of vaccine candidates with the potential to provide clinical benefit.

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