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"Current Dengue Vaccine Status"

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Abstract As the impact of dengue expands, a safe and effective vaccine will benefit the world greatly. Recent progress and novel approaches have advanced the field, but many challenges and unanswered questions remain for dengue vaccine developers. The most advanced dengue vaccine candidate, a chimeric yellow fever dengue (CYD) tetravalent vaccine, was the first dengue vaccine tested in a field efficacy trial. Several live-attenuated and inactivated vaccine candidate utilizing different antigen-presenting methods and adjuvants are currently in clinical trials. This manuscript highlights results of recent dengue vaccine studies, describes ongoing trials, and discusses future possibilities.

Keywords Dengue vaccine · Dengue serotype · Sanofi Pasteur chimeric yellow fever dengue vaccine · NIH-directed mutagenesis dengue vaccine · WRAIR GSK dengue purified inactivated vaccine · Merck recombinant envelope protein dengue vaccine · Takeda chimeric dengue vaccine · DNA dengue vaccine · Viral tropical medicine

Introduction

Dengue has been the most rapidly spreading mosquito-borne viral disease over the past 50 years, with an estimated 30-fold increase in annual cases. The World Health Organization estimates 50–100 million dengue cases per year, with 2.5 billion people living at daily risk of infection [1]. A more recent analysis estimates there are ~390 million dengue infections annually, with ~96 million being symptomatic [2•]. Dengue imposes a tremendous economic burden in endemic developing countries. Dengue is a significant cause of systemic febrile illness in returning travelers, and there is a long history of dengue negatively impacting deploying military personnel [3, 4]. There is an acute need for a dengue vaccine and the need will only increase as uncontrolled population growth persists, ecological change favors expanding vector habitats, and the ease of international travel continues.

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Dengue Vaccine Development Challenges

Currently, there are no licensed therapeutic dengue anti-virals. However, there are evidence-based treatment algorithms with excellent outcomes in facilities with dengue-experienced clinicians [5]. Without a licensed dengue vaccine, preventive strategies include personal protective measures and vector control. The implementation of personal protective measures has been difficult to achieve. The lack of sustained financial resources, community 'ownership', and expert leadership has hurt vector control programs [6]. For all these reasons, experts agree a safe and efficacious tetravalent dengue vaccine is the best option and hope for reducing the global dengue burden.

Dengue vaccine development faces several challenges due to the complex biology and epidemiology of the virus. Unlike other flaviviruses for which vaccines exist (Yellow fever, Japanese encephalitis, tickborne encephalitis), the dengue viruses (DENVs) exist as four antigenically distinct DENV serotypes (DENV-1, DENV-2, DENV-3, DENV-4). As a result, a dengue vaccine should offer protection against all four DENV serotypes. There are a few reasons for this. First, each type is capable of causing disease and death. Second, the predominating DENV serotype circulating in a given area is unpredictable from year to year, and one or more DENV serotypes often co-circulate at any given location [7–9]. Third, after infection with one DENV serotype, durable monotypic immunity develops against the infecting DENV type, while heterotypic, cross-reactive immunity (to other DENV serotypes) is incompletely protective (i.e. disease modifying, attenuating) and wanes over a period of months

[10–12]. Persistence of non-neutralizing heterotypic antibodies in conjunction with cross-reactive cellular immune responses substantially increases the risk of severe disease following a subsequent infection with a different DENV serotype [13–15].

A second development challenge is the lack of a validated animal disease model. Both mice and non-human primate (NHP) models have significant limitations. Mice, even 'humanized mice,' do not comprehensively mimic the human immune system, and as such, their responses to infection are only informative in a limited way. Non-human primates develop viremia and neutralizing antibodies after dengue infection, but they do not consistently develop clinical disease. Prevention of viremia in NHPs is an unreliable surrogate of clinical protective efficacy in a human. It is difficult to assess a vaccine candidate's potential for clinical benefit in preclinical development. [16–19].

The lack of an established correlate/surrogate of protection also complicates dengue vaccine development. Neutralizing antibody titers are an accepted marker of vaccine immunogenicity, but have yet to be correlated with protection from clinical disease endpoints [20]. Furthermore, the assay platforms currently used to measure neutralizing antibodies (i.e. plaque reduction neutralization test [PRNT], microneutralization) possess significant inter-assay and intra-assay variability, and critical assay reagents lack standardization [21, 22]. It is anticipated that efficacy studies will further elucidate the role of neutralizing antibodies and what constitutes a human immuno-protection profile. Cell-mediated immunity's role in immuno-protective and pathogenic immune responses is also under exploration [23–25]. It is likely that a single correlate of protection can be defined for all DENV serotypes, but surrogates (i.e. neutralizing antibody titer) will differ.

Basis of Dengue Vaccine Development

The DENV genome consists of a single-stranded, positive sense RNA that encodes three structural (capsid, premembrane/membrane, envelop) proteins and seven nonstructural (NS) proteins. The envelope (E) protein contains several serotype-specific neutralizing epitopes, and is considered a key antigen in vaccine development [16, 26]. Nonstructural protein one (NS1), may also contribute to type-specific protective immunity [27]. The other NS proteins (NS3, 4a, 4b, NS5) contain a majority of the CD4 and CD8 T cell epitopes [28]. The dengue vaccine candidate platforms discussed below represent unique approaches that strategically leverage a specific portion of the genome, based on the belief that an immune response to this portion will deliver a protective immune profile.

Chimeric Yellow Fever Dengue (CYD) Tetravalent Vaccine—Sanofi Pasteur

Currently in phase 3 clinical trials, Sanofi Pasteur's chimeric yellow fever dengue tetravalent dengue vaccine (CYD-TDV) is the most advanced candidate. Based on a yellow fever vaccine (YFV) 17D backbone, each vaccine strain of the live-attenuated dengue vaccine was achieved by substituting genes encoding the pre-membrane (prM) and envelop (E) proteins with those from a wild type DENV, one per DENV type. Four strains representing each serotype were combined to form the tetravalent vaccine. Phase 1 and phase 2 studies of the CYD-TDV given in endemic and nonendemic areas demonstrated acceptable safety in children and flavivirus primed volunteers (dengue natural infection, YF17D vaccination, or Japanese encephalitis vaccination). Expanded investigations failed to demonstrate immunologic evidence of enhanced disease. PRNT results showed a balanced neutralizing antibody response against all four DENV types after three doses in children and adults in all regions, regardless of flavivirus priming at enrollment [23, 29, 30].

Sanofi's first dengue vaccine efficacy study was conducted in school children in Ratchaburi province, Thailand [31••]. Among vaccine recipients, 91 % had anti-DENV or anti-JEV antibodies (PRNT50 titer > 10), and 70 % had dengue antibodies to one or more DENV types at baseline. When given in three doses at 0, 6, and 12 months, the CYD-TDV successfully boosted neutralizing antibody responses to all four DENV types in recipients. However, an analysis of clinical dengue cases revealed an overall vaccine efficacy of 30.2 %. Protection against DENV-2, the predominant circulating DENV serotype, was 9.2 % [31••]. The poor protection against DENV-2, despite similar PRNT50 titers to the other DENV types, raised several concerning issues. It is possible the DENV-2 strain genotype in the CYD vaccine did not protect against the circulating, genetically divergent DENV-2 Asian 1 genotype [17, 32]. The presence of neutralizing antibodies but failure to protect may indicate that neutralizing antibody is not the sole predictor of protection, or it may only be a relative correlate without guaranteed protection to some DENV types [33]. It is possible there was host immune interference following vaccination, and measured antibodies to DENV-2 were not type specific (homotypic), but were non-protective, cross-reactive antibodies [34, 35]. Sanofi Pasteur is currently conducting phase 3 clinical trials in Latin America (NCT 01374516) and Southeast Asia (NCT 01373281); results are expected by the end of 2014. A trial evaluating the immune profile of non-primed and JE vaccine-primed (U.S.) volunteers started in September 2013 and will encompass cell-mediated immunity and vaccine-induced viremia as endpoints (NCT 01943815). As of January 2013, over 28,900 human subjects have safely received the CYD-TDV [36].

Live Virus Vaccine Attenuated by Directed Mutagenesis, Chimeric (TetraVax)—U.S. National Institutes of Health (NIH)

The NIH used directed mutagenesis to develop an attenuated and immunogenic DENV-4 vaccine candidate [37]. The method of attenuation resulted in deletion of 30 nucleotides at the 3' untranslated region (UTR) of the dengue genome (rDEN4 Delta 30), and was applied to additional DENV types. Different monovalent candidates were evaluated in flavivirus-naïve adults to determine safety, replication kinetics, and immunogenicity [38]. Promising candidates were selected for formulation into a tetravalent admixture. Phase 1 studies in flaivirus-naïve adults identified TetraVax 003 (TV003) as the most promising admixture for further clinical development [39•]. TV003 contains DENV-1 and DENV-4 strains with delta30 deletions, a DENV-3 strain with delta30 and delta31 deletions, and a DENV-2/4 chimeric strain in which the DENV-2 prM and E proteins are inserted into a DENV 4 backbone. Initial studies in flavivirus-naïve volunteers demonstrated an excellent safety profile, with a faint, asymptomatic maculopapular rash being the most common post-vaccination examination finding. After a single dose, 45 % of the TV003 vaccinees generated a tetravalent antibody response (PRNT60>10) and 90 % had a trivalent response at day 42 post-vaccination. Seventy-five percent of those who received TV003 had measureable vaccine-induced peripheral viremia to at least one of the DENV types, as measured by viral isolation from blood during a 16-day post-vaccination period. DENV-3 virus was detected in 40 % of TV003 recipients, the highest among all serotypes [39•]. Preliminary reports of a phase 1 study in 56 flavivirus-primed (natural exposure or immunization) U.S. volunteers indicate that 85 % of TV003 vaccine recipients developed tetravalent neutralizing antibody responses after a single dose. Sixty (60 %) of subjects had at least one vaccine virus recovered after one vaccination, while none had detectable viremia after the 6-month booster dose [40]. Current development efforts include a phase 2 study in Brazil in partnership with the Butantan institute (NCT01696422), and a planned age de-escalation trial in Thailand in collaboration with the U.S. Army Medical Component-Armed Forces Research Institute of Medical Sciences and the Royal Thai Army Phramongkutklao Pediatrics Department.

Live Virus Vaccine Attenuated by Cell Culture Passage, Chimeric (DENVax)—Inviragen / Takeda

DENVax was developed by the U.S. Center for Disease Control and Prevention and adopted for further development by Inviragen (recently acquired by Tekada Pharmaceutical). A selected DENV-2 was attenuated by serial passage in primary dog kidney (PDK) cells, and is the backbone of each monovalent component of the tetravalent vaccine. Chimeras of the other three DENV serotypes were then created by substitution of the PrM and E genes of the attenuated DENV-2 strain with those of selected DENV-1, DENV-3, or DENV-4 strains [41•]. Phase 1 studies in flavivirus naive US and Colombia volunteers demonstrated candidate safety and tolerability. In the Colombia study, both high (subcutaneous (SQ) administration) and low doses (intradermal (ID) dosing) were well tolerated on a 0, 90-day vaccination schedule. The low dose ID route produced a higher percentage of tetravalent antibody seroconversion rates by PRNT compared to the high dose SQ route (71 % vs. 47 %) [42, 43]. It is possible ID dosing more closely resembles mosquito delivery of virus, resulting in more efficient antigen processing, and therefore superior immunogenicity [44]. The majority (83 %) of subjects had detectable DENV-2 viral RNA after the first dose, but none had detectable viral RNA after the second dose [42]. The absence of viremia following the second dose could be due to the fact that the first dose elicited an immune response that 'protected' the subjects for the second dose [10]. Phase 2 studies in Puerto Rico, Columbia, Thailand and Singapore inclusive of flavivirus-primed individuals over a broad age range are ongoing. Additional studies continue to evaluate dose compression and route (ID, intramuscular) and method (needle, needleless injector) of delivery (NCT01511250, NCT01765426).

Live Virus Vaccine Attenuated by Cell Culture Passage—Walter Reed Army Institute of Research (WRAIR) / GlaxoSmithKline Vaccines (GSK)

The WRAIR and GSK co-developed tetravalent vaccine candidates by combining DENV-1, DENV-2, DENV-3, and DENV-4 viruses attenuated by serial passage in PDK cells. Phase 1 studies identified monovalent candidates for inclusion in tetravalent formulations and tetravalent candidates with optimal balance of safety and immunogenicity [45]. A single formulation was advanced for testing in flavivirus-naïve toddlers and children in Thailand. Two vaccine doses administered on a 0 and 6 month schedule were well tolerated and immunogenic [46, 47]. A third dose of vaccine in the Thai studies demonstrated no sustainable immunogenicity impact (Thomas, S., personal communication).

To support phase 2 testing, the vaccine candidate was re-derived and a new candidate formulation produced with a lower DENV-4 viral concentration. Studies were conducted in flavivirus-primed and naïve U.S. and Thai adults and across a broad age range (12 months – 50 years). Vaccination was well tolerated with an acceptable safety profile in all groups in

all studies. Tetravalent seroconversion rates in flavivirus naïve, U.S. volunteers after two doses administered on a 0 and 6 month schedule ranged from 60 % to 66.7 % [48]. Instances of DENV-4 viremia were less among those who received the re-derived vaccine candidate (one subject, flavivirus naïve, asymptomatic) compared to the non-re-derived candidate (five subjects, four were flavivirus naïve, four with symptomatic viremia). During the follow up phase, physical examinations, clinical laboratory determinations, and lack of symptomatic viremia all support the acceptable safety profile of the new vaccine candidate [48]. In the Thai study, asymptomatic, low level viremia (DENV-2, DENV-3 or DENV-4) was detected in five of 80 vaccine recipients. A 97.1 % tetravalent seroconversion rate was measured one month post-dose 2, compared to a maximum of 78.9 % measured at baseline [49], Watanaveeradej, V. et al., AJTMH, in press).

The WRAIR and GSK have halted development of these candidates in pursuit of an improved target product profile (i.e. shorter dosing interval, shorter time to protection, etc.).

Purified Inactivated Virus Vaccine—Walter Reed Army Institute of Research / GlaxoSmithKline Vaccines

The first highly purified, formalin-inactivated virus (PIV) vaccine was developed at WRAIR in the mid-1990s. An inactivated DENV-2 candidate formulated with aluminum hydroxide (AlOH) produced neutralizing antibody responses in mice and rhesus macaques [50]. The remaining DENV types were produced and tetravalent vaccine candidates (DPIV) formulated with alum and GSK proprietary Adjuvant Systems (AS). Two doses of DPIV adjuvanted with AlOH or GSK AS and administered to non-human primates (NHP) 28 days apart were moderately to highly immunogenic, as measured by neutralizing antibodies. Neutralizing antibody persisted for at least 40 weeks and provided near-complete "protection" against DENV-2 challenge [51]. The most promising formulations were advanced to clinical testing.

Two phase 1 studies are ongoing, testing the safety and immunogenicity of DPIV adjuvanted with alum, AS01E, and AS03B. One hundred subjects have been enrolled at trial sites in the U.S. and Puerto Rico (NCT01666652, NCT01702857) In the U.S. study, 47, 88, 100, and 94 % of subjects who received two doses of 1ug-AlOH, 4ug-AlOH, 1ug-AS01E, and 1ug-AS03B, respectively, seroconverted (neutralizing antibody) against all four DENV types at Day 56. Geometric mean neutralizing antibody titers (GMTs) induced by the 1ug-AlOH formulation against DENV-1, DENV-2, DENV-3, and DENV-4 were 40, 102, 73, and 34, respectively. The 4ug-AlOH formulation induced GMTs of 253, 354, 208, and 83; 1ug-AS01E GMTs were 516, 778, 760, and 106; and 1ug-AS03B 883, 567, 590, and 104 [52], Schmidt A., et al., abstract, ASTMH 16 Nov 2013).

Recombinant Envelope (E) Protein—Merck

Merck is developing a recombinant subunit dengue vaccine candidate comprised of 80 % of the DENV E protein from each DENV type. The E proteins are expressed and produced in Drosophila S2 cells. Adjuvanted (ISCOMATRIX) and unadjuvanted tetravalent formulations were tested in NHPs, demonstrating a balanced neutralizing antibody response. NHPs were protected from challenge [53]. The V180 candidate mixed with alhydrogel or ISOCOMATRIX is currently in phase 1 clinical testing [54].

DNA Based Platforms—Naval Medical Research Center (NMRC)

A D1ME100 vaccine was developed by the NMRC using a plasmid vector to express the preM and E genes using a human cytomegalovirus promoter [55]. In a phase 1 clinical trial, the vaccine was administered intramuscularly via Biojector at 0, 1, and 5 months in a dose-escalation study design. The safety profile was acceptable, but there were poor neutralizing antibody responses with five of 12 (41.6 %) high-dose (5 mg) recipients developing antibody titers above the assay cutoff [56]. Applying this same technology, a tetravalent DNA dengue vaccine candidate was formulated. The lipid-based adjuvant Vaxfectin (Vical incorporated) induced anti-dengue neutralizing antibodies against DENV-1, DENV-3, and DENV-4 in non-human primates. Shorter periods of viremia were seen in NHP vaccinated with the adjuvanted product after DENV 2 virus challenge [57]. A phase 1 human trial comparing the DNA tetravalent vaccine adjuvanted with two doses of vaxfectin administered at 0, 30, and 90 days has completed enrollment, and results are expected soon (NCT01502358).

Future Directions

The dengue vaccine development field is robust, with a diverse portfolio of vaccine candidates and constructs in clinical development. Numerous additional candidates and approaches are being explored in preclinical development activities. Replicating, non-replicating, full or partial dengue virus genome constructs, use of adjuvants and vector systems, and heterologous prime boost administration strategies are all being studied [58].

There is much to learn about the DENVs and the complex immunologic interactions that occur between virus and the human host. The repertoire of immunology assays being employed in dengue vaccine development pathways has expanded significantly as it has become clear that deconstructing

and recreating immunoprotective immune profiles is extremely challenging. Developers continue to measure anti-DENV neutralizing antibodies using the classic plaque reduction neutralization test (PRNT), while others are performing microneutralization assays and others are developing new platforms [59]. More recent discussions have focused not only on the testing platform, but on the optimization of critical reagents (i.e. cell lines) and testing conditions with the intent of improving the physiologic relevancy of the readout [60–62]. Considerable effort and advancement has been made in the measure of cellular mediated immune (CMI) responses following vaccination with experimental dengue vaccine candidates [63–65]. A key deficiency in characterizing dengue vaccine immunogenicity is the ability to identify, quantify, and qualify DENV-type–specific, homotypic immune responses to vaccination from cross-reactive, heterotypic, non-protective, and perhaps enhancing, responses.

Finally, in the absence of an animal disease model and immune correlate of protection, a dengue human infection model (DHIM) may be a valuable tool for determining a vaccine candidate's viability early in its development. Correlates of protection could also be explored using DHIM. Regulatory strategies, and licensing and labeling of dengue vaccine candidates may be influenced by a well-characterized and consistently performing human infection mode [17, 27, 66•, 67].

Conclusion

A safe and efficacious tetravalent dengue vaccine would benefit billions of people living in dengue endemic areas, millions of travelers to these areas, and deploying military personnel. Numerous experimental candidates are in preclinical and clinical testing in government, academic, and industry research and development programs. Significant gaps in our understanding of dengue immunology have challenged the field. New and improved animal models, immunologic assay platforms, and data from prospective field studies are informing dengue vaccinologists and offering hope that licensure of a first generation dengue vaccine is not too far in the future.

Compliance with Ethics Guidelines

Disclaimer The opinions or assertions contained herein are the private views of the authors and are notto be construed as reflecting the official view of the United States Army or the United States Department of Defense.

Conflict of Interest Leyi Lin and Stephen Thomas declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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