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Dengue vaccine development by the year 2020: challenges and prospects

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The first licensed dengue vaccine led to considerable controversy, and to date, no dengue vaccine is in widespread use. All three leading dengue vaccine candidates are live attenuated vaccines, with the main difference between them being the type of backbone and the extent of chimerization. While CYD-TDV (the first licensed dengue vaccine) does not include non-structural proteins of dengue, TAK-003 contains the dengue virus serotype 2 backbone, and the Butantan/Merck vaccine contains three full-genomes of the four dengue virus serotypes. While dengue-primed individuals can already benefit from vaccination against all four serotypes with the first licensed dengue vaccine CYD-TDV, the need for dengue-naive population has not yet been met. To improve tetravalent protection, sequential vaccination should be considered in addition to a heterologous prime-boost approach.

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Introduction to dengue vaccine development

Dengue vaccine development has been hampered and delayed by remarkable challenges. The four genetically succinct but still closely related dengue serotypes are known to interact immunologically with potential for disease enhancement. As a tetravalent immune response is desired, when given a mixture of all four serotypes in a tetravalent live-attenuated vaccine, each component would need to independently result in four different monotypic immune responses that are solid to each serotype. This has unfortunately proven to be difficult to achieve. Immune correlates to predict protection versus disease enhancement are still lacking [1], and plaque reduction neutralization assays do not reliably differentiate between serotype-specific versus heterotypic antibodies [1,2•]. Other challenges include the lack of a reliable animal model. Furthermore, dengue is primarily a disease of low and middle income countries, thus dengue research often does not receive the level of funding needed to accelerate vaccine development [3]. Unsurprisingly then, it has taken several decades to develop a vaccine. The first licensed dengue vaccine led to considerable controversy [4], and to date, no dengue vaccine is in widespread use.

Nevertheless, we need to press on. Dengue was identified as one of the 10 threats to global health in 2019 by WHO, underlining the urgent need for a vaccine. The primary need for a dengue vaccine as a public health tool is the unpredictable nature of dengue outbreaks overwhelming already existing fragile health care systems, the extremely high annual incidence of at least 100 million cases and the epidemic trajectory which shows a relentless increase over the past two decades [2•,5,6]. Dengue infections in the communities, and even hospitalized dengue, lead to inappropriate antibiotic use in more than 30%of cases [7]. Dengue has also become a leading problem in international travelers [8–12]. Certain risk factors are predictive of more severe disease outcome such as young or old age, prior dengue infection, diabetes, sickle cell disease and underlying medical conditions [2•,13].

Vaccine candidates

All three leading dengue vaccine candidates are live-attenuated vaccines, with the main difference between them being the type of backbone and the extent of chimerization.

First licensed dengue vaccine

CYD-TDV, a tetravalent live attenuated with a yellow fever 17D backbone, is the first dengue vaccine to be licensed, under 'Dengvaxia'. Despite being first licensed in 2015 in Mexico followed by 20 other dengue endemic countries based on results from Phase 3 trials conducted in more than 30 000 children and adolescents aged 2−16, it was only introduced in two subnational public health programs in the Philippines and Brazil. The Phase 3 trials revealed a vaccine efficacy that differed by age, serostatus and serotype. In terms of cumulative incidence, CYD-TDV showed a population level benefit [14]. Further post-hoc retrospective analyses of the long-term safety data revealed an excess risk of severe dengue in those who were seronegative at baseline. Serostatus refers to whether a person has had dengue infections in the past [15••]. This increased risk in seronegative subjects was observed starting from 30 months after administration of

the first dose. A plausible hypothesis is that CYD-TDV may trigger an immune response to dengue in seronegative persons that predisposes them to a higher risk of severe disease, analogue to what is seen in natural secondary dengue infections [16]. A subsequent infection with the first true wild type dengue virus would then be a 'secondary-like' dengue illness. Dengue non-structural proteins (NS) are absent from the Sanofi dengue-yellow fever chimeric vaccine. Given that NS1 may have toxin-like properties that disrupt the endothelial glycocalyx through either inflammatory-dependent or independent pathways [17–20], the absence of NS1 in CYD-TDV could also be a potential explanation for the limited vaccine performance.

The World Health Organization (WHO) recommends that for countries considering CYD-TDV vaccination as part of their dengue control program, a pre-vaccination screening strategy, in which only dengue-seropositive persons are vaccinated, is the recommended strategy [21]. In May 2019, the US Food and Drug Administration (FDA) approved CYD-TDV for use in seropositive individuals 9 through 16 years of age living in endemic areas of the U.S. The European Medicine agency also endorsed the use of this vaccine in seropositive individuals with a wider age range.

The programmatic use of CYD-TDV, therefore, requires screening for serostatus before vaccination. IgG-based enzyme-linked immunosorbent assays (ELISAs) or rapid diagnostic tests (RDTs) can be used for pre-vaccination screening, also called a 'test and vaccinate' strategy. Ideally, a screening test should be both highly sensitive and specific to minimize false positives and negatives to yield maximal population level benefit and minimize harm by correctly screening for seropositive individuals only [22]. It should also be affordable, simple to use and provide rapid results. Two recent comparative evaluations on currently available assays showed high specificity (>98%) for all immunoassays apart from one RDT, but variable sensitivities (higher sensitivities observed for the ELISAs [89% and 93%] than the RDTs [48–71%]) [23•,24•] Sensitivity appeared similar in samples from individuals with recent and remote virologically confirmed dengue (VCD). Cross-reactivity to other flaviviruses was low with RDTs (</=7%), but more significant with ELISAs (up to 51% for West Nile and 34% for Zika).

CYD-TDV's public health utility is limited to seropositive persons. Implementation research is now needed on how and for which settings (e.g. school settings) a pre-vaccination screening can be rolled out for programmatic national or subnational use [25]. In private clinics and travel medicine settings [26], blood is often taken before hepatitis B vaccination to check for hepatitis B serostatus and other vaccine-preventable diseases; thus there is precedence for pre-vaccination screening. Research is also needed to evaluate vaccine schedules with fewer doses, assess the need and timing for booster doses, and identify populations that will benefit most from this vaccine [21,27,28].

Lessons from the first licensed dengue vaccine for clinical trial designs

Lessons from the first dengue vaccine have shaped how second-generation dengue vaccines should be evaluated. The effect on cellular immunity needs to be studied, including the extent of truly neutralizing antibodies versus just transient cross-protective antibodies. Vaccine trial designs should account for the known period of cross-protection between serotypes which can last up to one even two years before safety signals may appear in year 3 and beyond. Trial designs, therefore, need to be extended to include active surveillance of trial participants up to 3−5 years [29]. Furthermore, vaccine evaluation must include a-priori analysis plans for stratification by serostatus and serotype. To stratify by serostatus, baseline blood samples need to be taken from all study subjects. When interpreting efficacy results, one has to consider the predominance of a given serotype and the proportion of individuals who are seronegative in a population, which can vary from year to year and country to country [30]. Indeed, the vaccine trials for the second-generation live dengue vaccines take these trial specifications into account.

Dengue vaccines in Phase 3 trials

Non-structural proteins of the dengue virus backbone are at least partially present in second generation dengue vaccines. Two chimeric live-attenuated dengue vaccines are now in Phase 3 trials: one developed by Takeda (TAK-003) and one by the National Institute of Allergy and Infectious Diseases (TV003/TV005) (Table 1).

(1) Takeda's TAK-003

Takeda's live-attenuated tetravalent dengue vaccine candidate comprises an attenuated DENV-2 strain plus chimeric viruses containing the prM and E genes of DENV-1, DENV-3 and DENV-4 cloned into the attenuated DENV-2 'backbone' [31]. The difference to Dengvaxia therefore is the presence of non-structural proteins due to the DENV2 backbone. TAD-003 induces cross-reactive T cell-mediated responses that may be necessary for broad protection against dengue fever [31,32]. In agreement with WHO's prequalification requirements for dengue vaccines, Takeda has manufactured a lyophilized formulation of TAK-003 that allows stable storage at +2 degrees C to +8 degrees C. In a randomized, double-blind, phase 2 study (NCT02193087) in 1002 healthy dengue-naive adults, 18–49 years of age, GMTs and

Table 1

Comparison of the 3 leading tetravalent live-attenuated dengue vaccine candidates

	CYD-TDV (Dengvaxia) (Sanofi Pasteur)	TAK-003 (Takeda)	TV003/TV005)/ NIH)
Licensure	December 2015	Not yet	Not yet
Phase 3	Completed 5 year observation time	Stage 1 and 2 completed and published	Recruitment closed, Phase 3 trial results not yet published
Study sites of Phase 3 trial	10 countries in Asia and Latin America	8 countries in Asia and Latin America	Brazil
Age range of Phase 3 study 2–59	participants	2–16	4–16
Doses	3 (6 months apart), but label change is imminent for 2 doses only	2 doses (3 months apart)	1 dose
Backbone	Yellow fever	DENV 2	Full-genome for DENV 1, 3 and 4. Backbone for DENV 2 is DENV 4
Dengue non-structural proteins	Not present in the vaccine construct	DENV 2	DENV 1, 3 and 4

seropositivity rates to all four serotypes were achieved [33]. A Multi-Color FluoroSpot (MCF) assay enabled quantitation of serotype-specific and cross-reactive individual memory B cells (MBCs) secreting DENV-specific antibodies in a polyclonal mixture [34]. Using the MCF assay, type-specific and cross-reactive MBC responses were investigated; the results demonstrate that, unlike primary or secondary natural DENV infection, tetravalent vaccination elicits tetravalent type-specific MBCs, and thus all four components of TAK-003 contribute to the DENV-specific MBC response following vaccination [33].

The Phase 2 trials on immunogenicity showed that geometric mean titers (GMTs) against DENV 1, DENV 3, and DENV 4 were lower in participants who were seronegative and receiving one primary dose than in those who received the two-dose primary series or one primary dose plus a 1-year booster [30]. These immunogenicity results suggested that Phase 3 trials should be conducted with a two dose regimen to improve immunogenicity in seronegative individuals. At Month 36, seropositivity rates were 97.3%, 98.7%, 88.0% and 56.0% for DENV-1, DENV-2, DENV-3 and DENV-4, respectively [35••].

A Phase 3 trial in more than 20 000 healthy children and adolescents 4–16 years of age to receive two doses of vaccine or placebo is currently being conducted in 8 countries in Asia and Latin America.

Part 1 Phase 3 trial over a time period of 12 months: The overall vaccine efficacy in the safety population was 80.9% (95% confidence interval [CI], 75.2–85.3; 78 cases per 13 380 [0.5 per 100 person-years] in the vaccine group versus 199 cases per 6687 [2.5 per 100 person-years] in the placebo group) [36]. In the per-protocol analyses, vaccine efficacy was 80.2% (95% CI, 73.3–85.3; 61 cases of virologically confirmed dengue in the vaccine group versus 149 cases in the placebo group), with 95.4% efficacy against dengue leading to hospitalization (95% CI, 88.4–98.2).

Part 2 Phase 3 [37••] data up to 18 months post-vaccination (NCT02747927) reported an overall vaccine efficacy of 80.2% (95% CI 73.3–85.3; 61 cases of VCD in the TAK-003 group versus 149 cases of VCD in the placebo group). In the secondary endpoint assessment timeframe, an overall vaccine efficacy of 73.3% (95% CI 66.5–78.8) was observed. Analysis of secondary endpoints showed efficacies of 76.1% in individuals who were seropositive at baseline, 66.2% in individuals who were seronegative at baseline, 90.4% against hospitalized dengue, and 85.9% against dengue haemorrhagic fever. Efficacy varied by individual serotypes: DENV 1, 69.8%, DENV 2, 95.1%; DENV 3, 48.9%; DENV 4, 51.0% [−69.4–85.8]).

In summary, although the Takeda vaccine appears much less serostatus dependent compared with CYD-TDV and efficacy data look promising, some complex nuances for serotypes 3 and 4 will require extended follow-up, and careful balancing by regulators and policy makers in determining the potential utility and safety of this vaccine [30].

(2) National institute of allergy and infectious diseases (TV003/TV005)/Butantan

This vaccine comprises 3 full-length DENV attenuated by one or more deletions in the 3′ untranslated region, while the fourth component is a chimeric virus in which the prM and E proteins of DENV-2 replace those of DENV-4 in the DEN4Δ30 background [38]. Thus, this vaccine carries the full-genomic backbone of three dengue serotypes, except for DENV2. The capacity to elicit CD4+ cell responses closely mirrors those observed in a population associated with natural immunity [39]. A single-dose induces robust tetravalent antibody and cellular T cell responses and resulted in a 100% efficacy in a human challenge study [40]. Developed by the U.S. National Institutes of Health (NIH/NIAID), it is currently in a Phase 3 trial in Brazil through Butantan, but was also licensed to Merck for further development outside of Brazil. The Butantan Institute has

manufactured a lyophilized tetravalent live-attenuated dengue vaccine Butantan-DV, which is analogous to the US National Institutes of Health (NIH) TV003 admixture [30]. Seroconversion appears to be high for all four serotypes independent of serostatus, with the highest for DENV 1, 3 and 4, and the lowest for DENV 2.

To determine the ability of a single dose of the live attenuated tetravalent dengue vaccine TV003 to induce a suitable neutralizing antibody response, a placebo-controlled clinical trial was performed in 48 healthy adults who received 2 doses of vaccine or placebo administered 12 months apart. Evaluation of safety, vaccine viremia, and neutralizing antibody response indicated that a single dose is sufficient [41].

Thus, this vaccine has gone into Phase 3 trial with a single dose (in contrast to TAK-003 with 2 doses, and CYD-TDV with 3 doses). The Phase 3 results remain unpublished to date, without known interim analyses.

Dengue vaccines for travelers

Given that many endemic countries are popular tourist destinations, international travelers are increasingly at risk of dengue [5,8,10–12,42], with attack rates reported as high as 5.51 cases per 1000 travel-months [43]. Dengue is a frequent problem in travelers [44.45], more frequent than 'traditional' travel-associated infectious diseases such as typhoid fever [46], rabies [47], and yellow fever [48,49]. GeoSentinel is an international network of travel medicine providers to monitor trends in travel-associated diseases [50–52] which has reported a substantial increase of dengue over the past decade [53]. Dengue can affect tourist travelers, business travelers and expatriates [12,52], migrants including those visiting friends and relatives (VFR) [54], and pilgrims [55]; both in adult and pediatric travelers [11,42,56]. Interruption of travel, hospitalization during or after travel, and out-of-pocket expenses can ensue [9]. Dengue is now much more frequent than many of the other travel-associated vaccine preventable diseases such as rabies, hepatitis A [57], yellow fever or Japanese encephalitis [58,59]; thus, vaccination against dengue would be an indication in the travel medicine context. The limitation of the currently only licensed dengue vaccine, CYD-TDV, is that it should only be used in seropositive travelers [27]. However, most travelers are seronegative. Furthermore, the dosing schedule of 3 doses 6 months apart for CYD-TDV renders the use of such a vaccine difficult in the travel medicine setting. A safe and efficacious vaccine that can be used regardless of serostatus is needed for travelers [60]. Until a vaccine becomes available that would benefit all travelers to dengue endemic countries, travelers should be advised to take day-time personal protective measures against mosquito bites [61] and consider Dengvaxia if they are seropositive [28]. Pre-travel advice for all travellers to dengue endemic countries need to include advice on the dengue risk [5,62].

Potential solutions to overcome viral interference for tetravalent live attenuated dengue vaccines

While dengue-primed individuals can already benefit from vaccination against all four serotypes with the first licensed dengue vaccine CYD-TDV, the need for dengue-naive population has not yet been met. Would sequential immunization induce stronger and broader immunity against four DENV serotypes than tetravalent-formulated immunization and overcome the viral interference we have seen to date for the live attenuated dengue vaccine formulations? In a study in Singapore mice were immunized with four DNA plasmids, each encoding the pre-membrane and envelope from one DENV serotype, either sequentially or simultaneously. The sequential immunization induced significantly higher levels of interferon (IFN)γ-expressing or tumor necrosis factor (TNF)α-expressing $CD4^+$ and $CD8^+$ T cells to both serotype-specific and conserved epitopes than tetravalent immunization [63]. Moreover, sequential immunization induced higher levels of neutralizing antibodies to all four DENV serotypes than tetravalent vaccination. In these animal data, sequential immunization resulted in more diversified immunoglobulin repertoire, and suggests that sequential immunization offers an alternative approach to potentially overcome the current challenges encountered with tetravalent-formulated dengue vaccines.

Another strategy to overcome viral interference for tetravalent dengue vaccines would be to use a heterologous prime-boost strategy. While TAK-003 vaccine induces high protection against DENV2 and to a lesser extent against DENV1 in both dengue-seropositive and —seronegative individuals, CYD-TDV induces a high protection against DENV3 and 4 but to a lesser extent against DENV1 and 2. Furthermore, while TAK-003 vaccine performance seems to be less serostatus dependent, an inconclusive relative risk >1 has been observed for DENV3 in seronegative vaccinees and no conclusion could be drawn regarding DENV4 [30]. So what about combining both vaccines in a heterologous prime-boost regimen, leveraging upon the benefits of each vaccine and thereby minimizing safety concerns? Priming with TAK-003 followed by a CYD-TDV boost would initially ensure strong humoral and cellular responses against DENV2 — the weakest CYD-TDV serotype —, and then eventually strengthen responses against the other serotypes, in particular DENV4 — the dominant CYD-TDV and weakest TAK-003 serotype [64••]. Heterologous CYD-TDV boost may also likely induce broader cross-reactive immune responses at both humoral and cellular levels. Moreover, CYD-TDV possesses an YF-17D backbone, decreasing the risk of being negatively impacted by initial TAK-003-

induced DENV2-specific cellular responses. A DENV 1-2 dominant vaccine followed by a DENV3-4 dominant vaccine may also better reflect the theoretical advantages of sequential infections as outlined above. It is likely that Takeda will only investigate such a prime-boost strategy after their vaccine has been licensed. Combining vaccine platforms developed by competing companies may pose challenges, but these can be overcome.

Other dengue vaccine candidates

Next-generation dengue vaccines in development include DNA, subunit, virus-like particles (VLP) and viral vector vaccines [65]. Two phase I clinical trials were conducted to evaluate the safety and efficacy of the tetravalent formulation purified inactivated vaccine combined with different adjuvants (e.g. aluminium hydroxide, AS01E or AS03B) [66,67]. All formulations were well tolerated and induced a balanced immune response against all four serotypes, with the highest mean antibody titers reached with AS01E and AS03B. A phase 2 trial is currently evaluating a tetravalent purified inactivated dengue vaccine with AS03B to determine the most effective injection schedule (0-1, 0-1-6, or 0-3 months) (NCT02421367)

Challenges to dengue vaccine development in an era of other emerging viral diseases

The emergence of Zika virus as a public health problem of international concern in early 2016, a vector-borne virus with close genetic similarity to dengue viruses, was the first challenge to dengue vaccine development, followed by the emergence of another virus by late 2019, not related to dengue, SARS-CoV-2 causing coronavirus related disease (COVID-19). Although dengue virus is not associated with severe pregnancy outcomes as Zika [68•], not thought to be sexually transmitted [69,70] and not as strongly associated with neurological complications such as Guillain-Barre Syndrome [71], the emergence of Zika has complicated dengue vaccine development because of the potential immunological interaction between these closely related viruses. By acquiring cytotoxic T-cell epitope-rich regions from Culex-borne flaviviruses, ZIKV evaded DENV-generated T-cell immune cross-protection [72]. Interestingly, pre-existing dengue immunity has minimal impact on the innate immune response to Zika [73]. Primary and secondary DENV elicit similar memory B-Cell responses, but breadth to other serotypes and cross-reactivity to Zika virus is higher in secondary dengue [74•]. Immunity to DENV only modestly shapes breadth and magnitude of enduring ZIKV antibody responses [75••]. While the evidence is mounting that preexisting high antibody titers to dengue virus were associated with reduced risk of ZIKV infection and symptoms [76], there is still lack of data on whether pre-existing immunity to Zika protects against or enhances a subsequent dengue infection. These are data gaps that need to be addressed for dengue vaccine development. Clearly, the presence of co-circulating arboviruses such as dengue and Zika increases the chance of co-infection and demonstrates the importance of the differential diagnosis, especially during periods of arboviral outbreaks [77], and this needs to be taken into consideration for clinical trial design.

The current COVID-19 pandemic has placed immense pressure on health care and public health systems worldwide. COVID-19 and dengue co-infections have been reported [78]. The response to this pandemic unfortunately has diverted resources and finances; and pushed dengue vaccine development out of the international spotlight. A resurgence of dengue is a real threat during the COVID-19 pandemic because the high burden of dengue related hospitalizations will further overwhelm already overwhelmed healthcare systems [79]. The COVID-19 pandemic therefore provides even more impetus to develop, license and roll out dengue vaccines for broader use.

Summary and outlook

The first licensed dengue vaccine led to considerable controversy, and to date, no dengue vaccine is in widespread use. All three leading dengue vaccine candidates are live-attenuated vaccines, with the main difference between them being the type of backbone and the extent of chimerization. While CYD-TDV (the first licensed dengue vaccine) does not include non-structural proteins of dengue, TAK-003 contains the dengue virus serotype 2 backbone, and the Butantan/Merck vaccine contains three full-genomes of the four dengue virus serotypes. The four genetically succinct but still closely related dengue serotypes are known to interact immunologically with potential for disease enhancement. While dengue-primed individuals can already benefit from vaccination against all four serotypes with the first licensed dengue vaccine CYD-TDV, the need for dengue-naive population has not yet been met. To improve tetravalent protection, sequential vaccination should be considered in addition to a heterologous prime-boost approach. The ideal properties of a dengue vaccine should include the ability to induce long-lasting homotypic immune responses to all four serotypes in all age groups, regardless of dengue serostatus. The vaccine should have a schedule ideally with 2 or fewer doses, should be able to prevent dengue outbreaks if used early at the onset of the outbreak, and should serve as prophylaxis in large populations to effectively prevent epidemics in the long term.

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