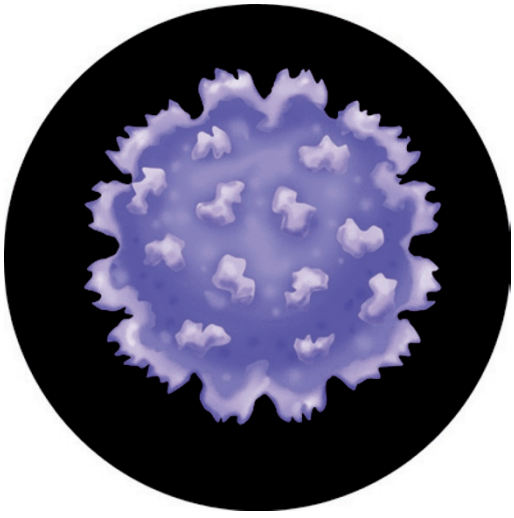
Defeating dengue: a challenge for a vaccine

Dengue poses a substantial clinical problem, with the four serotypes of dengue viruses infecting 50–100 million people per year and approximately half the world's population estimated to be at risk. As there is no specific treatment for dengue, the development of an effective vaccine will be an important measure for controlling this disease. A phase 2b trial testing a recombinant live, attenuated tetravalent dengue–yellow fever chimeric virus vaccine in Thai schoolchildren of 4–11 years of age has recently been reported[1]. The vaccine showed an overall efficacy of 30.2%, and one or more doses of the vaccine reduced the incidence of disease caused by the DENV3 and DENV4 dengue virus serotypes by 80–90%, with less reduction of disease caused by DENV1; however, there was no efficacy against DENV2. We asked three experts to comment on the results of this trial and how they might influence the landscape of dengue vaccine development.



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There are four serotypes of dengue virus, and infection with one serotype induces adaptive immune responses that are believed to provide durable immunity against re-infection by the same serotype. However, a second infection with a different serotype increases the relative risk of developing severe disease, possibly because poorly neutralizing cross-reactive antibodies from the primary infection enhance infection of the second dengue virus serotype in cells expressing Fcγ receptors[2]. Thus, the goal of dengue virus vaccine development is to produce a balanced protective antibody response against all four serotypes and avoid an incomplete immune response that theoretically could facilitate pathogenesis[3].

The much-anticipated results of a phase 2b trial with a live, attenuated tetravalent dengue virus vaccine show that, surprisingly, the vaccine prevented only 30.2% of febrile dengue virus infections[1]. Although the incidence of DENV3 and DENV4 cases was reduced by 80–90%, the vaccine was less effective (~60%) against DENV1 and had no efficacy against DENV2. The low overall efficacy was skewed by a high number of DENV2 infections during the trial.

Why was there a lack of efficacy of the vaccine against DENV2 although neutralization titers were higher against DENV2 compared to DENV1 and DENV3? The authors suggest that future work should measure the neutralizing activity of serum on myeloid cells (which are dengue virus targets *in vivo*) that express Fcγ receptors instead of the currently used fibroblast cells[1]. Other possible explanations could be that the neutralization assay used cannot ascertain whether there were four balanced serotype-specific responses compared to less protective cross-reactive responses; that the circulating DENV2 strain was of a different genotype than the DENV2 strain used for vaccine formulation and subsequent neutralization assays and,

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The four dengue virus serotypes (DENV1–4) cause the most prevalent arthropod-borne viral disease of humans worldwide, yet the mechanisms by which the host immune response provides either protection from or enhancement of a subsequent infection with a different dengue virus serotype are poorly understood, and this has been a major hindrance in dengue vaccine development. The existence of four distinct serotypes—in essence, four different viruses—complicates matters further, as exemplified by the fact that the tetravalent dengue vaccine did not protect against DENV2 in the first proof-of-concept dengue vaccine efficacy trial[1]. Various reasons may explain this lack of protection[1,5], and several crucial points emerge from the trial results.

First, there is an urgent need for more in-depth immunological studies of vaccine recipients as well as of natural dengue virus infections, using samples from prospective cohort studies from documented first, second and third dengue virus infections before a subsequent symptomatic versus inapparent dengue virus infection. Second, an integrated analysis of B and T cell responses is necessary that focuses on diverse effector functions

thus, may not have been inhibited efficiently by vaccine-induced antibodies; that the neutralization test used did not account for other factors that modulate antibody inhibition, including virus maturation and interactions with complement[4]; or that the induction of protective but non-neutralizing antibodies and the role of T cell responses were not considered.

"One final consideration is how much protection is needed to control epidemics of severe disease, which requires sequential infection by multiple serotypes."

Although the results of this trial[1] were unexpected, they provide an opportunity for establishing better immune correlates of protection. The challenge will be to determine which of the explanations above account for the failure of the vaccine against DENV2. One final consideration is how much protection is needed to control epidemics of severe disease, which requires sequential infection by multiple serotypes. Would a vaccine that reduces infection of three serotypes be sufficient? An ongoing phase 3 efficacy study with more than 30,000 subjects may provide answers to this question. Then, we can decide whether 75% is closer to half-full or half-empty.

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that may contribute synergistically to protection. These include the role of complement, the contribution of type-specific versus cross-reactive neutralizing antibodies, antibody-dependent cell-mediated cytotoxicity and viral inhibition, B cell magnitude and breadth, and the epitope repertoire, magnitude, frequency, human leukocyte antigen restriction and multifunctionality of $CD8^+$ T cell responses. Third, for neutralization assays to be predictive, a more relevant human monocytic cell line that is susceptible to infection by mature and partially immature dengue virus virions and that expresses Fcγ receptors, which can mediate antibody-dependent uptake of dengue virus immune complexes, should be used (for example, the, U937-DC-SIGN line)[6]. Fourth, it will be crucial to study the pre- and post-vaccination immune responses at the individual level in relation to outcome and thus design vaccine trials such that preimmunization blood samples from all subjects are available for future study.

In sum, the results from this proof-of-concept vaccine efficacy trial highlight the crucial need to improve understanding of the immune response to natural dengue virus infections and vaccine candidates and to identify robust correlates of protection.

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Dengue virus infections are a major cause of morbidity and severe illness in tropical regions. The development of a safe, effective vaccine to provide protection against severe dengue by any of the four dengue virus serotypes is an important goal. The risk for more serious infection is increased during secondary dengue virus infections[2], indicating that residual immune responses to the first infecting virus do not protect against other dengue virus serotypes and that cross-reactive immune responses sensitize some individuals for more serious illness by a second virus. Factors such as the host immune genotype, the numbers of preexisting cross-reactive T cells and the concentrations of cross-reactive antibodies probably contribute to this increased risk[7,8].

"Although higher serum concentrations of dengue virus-neutralizing antibodies correlate with a decreased risk of illness, such immune correlates do not necessarily reflect protective mechanisms."

These factors make the development of a safe and effective tetravalent dengue virus vaccine a complex challenge. The recent study by Sabchareon *et al.*[1] offers some promise for a live, attenuated chimeric tetravalent dengue virus. Their results showed that the vaccine induced only modest protection against DENV1, DENV3 and DENV4 and none against DENV2, the major cause of dengue illness during this study. Future sequencing of DENV2 isolates from ill study subjects should determine the relevance of the vaccine DENV2 to circulating DENV2. A single dose of a monovalent YFV–DENV2 vaccine has been shown to protect monkeys against wild-type DENV2 challenge[9]. Testing the tetravalent dengue virus described by Sabchareon *et al.*[1] for protection in nonhuman primates against a subsequent DENV2 challenge, analogous to this human clinical trial, may be warranted. Unlike other viral infections such as measles or mumps, dengue virus infections can occur in the presence of naturally acquired neutralizing antibodies[10]. Standard and research-based antibody assays using all available preinfection sera might provide insights into the failure of this tetravalent dengue virus to protect some individuals from dengue. Although higher serum concentrations of dengue virus-neutralizing antibodies correlate with a decreased risk of illness[10], such immune correlates do not necessarily reflect protective mechanisms. Further research into dengue vaccine development should be stimulated by the unexpected results of this trial[1], which is nevertheless an important step forward in the quest for a safe, effective dengue vaccine.

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