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Guidelines for clinical trials of dengue vaccine in endemic areas

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ABSTRACT

Guidelines for the clinical evaluation of dengue vaccines in endemic areas have recently been developed, building upon earlier recommendations published in 2002 (WHO. 2002. Guidelines for the evaluation of dengue vaccines in populations exposed to natural infection. Geneva, Switzerland. Report no. TDR/IVR/DEN/02.1). This new document discusses the rationale and background of dengue vaccine trials and outlines dengue case definitions, proposed efficacy end points, requirements for trial sites, methods of measurement and proposed safety schedules. Demonstrated protective efficacy against each of the four dengue virus serotypes without safety concerns is the objective of any candidate tetravalent vaccine clinical trial. Accurate epidemiological data of dengue and other circulating flaviviruses over multiple transmission seasons are required to address factors such as background flavivirus immunity and subclinical infections that may confound serological results. Furthermore, bridging and post-licensure studies may be necessary to extend conclusions concerning vaccine characteristics, while co-administration trials are necessary in paediatrics. These guidelines are primarily aimed at national regulatory authorities, vaccine developers and research scientists and should be analysed, discussed and adjusted where necessary.

1. Introduction

The *Guidelines for the clinical evaluation of dengue vaccines in endemic areas*,[1,2] have been developed with contributions and comments from many individuals from a variety of countries and institutions (see Appendix 1) with the aim of identifying the basic technical information required to design dengue vaccine field trials. The purpose of such trials is primarily to capture sufficient data on candidate vaccine safety and efficacy to support licensure, and secondarily, to establish its safety and long-term protection, in post-licensure field studies. It is a living document, to be revised in response to scientific advances. The guidelines are primarily intended for national health and regulatory authorities in dengue-endemic countries who are interested in vaccine development, and the implementation of field trials and dengue control programmes, as well as to vaccine developers and research scientists. The guidelines are not designed to provide guidance for introduction of dengue vaccines into national immunisation programmes.

2. Justification for development of guidelines

Dengue is a complex disease with a spectrum of clinical manifestations. Infection with any of four related flaviviruses, referred to as dengue types 1, 2, 3, and 4, can result in an acute febrile disease. Dengue fever (DF) is the most commonly diagnosed specific form, characterised by sudden onset of fever lasting between two and seven days, accompanied by severe headache, gastrointestinal symptoms, muscle, joint and bone pain, and a rash.[3,4] The classic form is self-limited and usually results in complete recovery. Dengue haemorrhagic fever (DHF) is a more severe manifestation; it is far less common than dengue illness, and criteria have been developed to classify its severity.[4] However, this classification is currently under review in a multicentre study. DHF has the same characteristics as DF in early stages, followed by haemorrhaging and/or increased vascular permeability in the later stages, which in turn may lead to vascular collapse, or 'dengue shock syndrome' (DSS), and death.

Individuals may only develop disease once with one specific dengue serotype, leading to lifelong homotypic protection against that serotype. However, there is little or no heterotropic protection following an original infection, and sequential heterologous infection is associated with risk of severe disease. These considerations have given rise to a number of hypothetical safety concerns, although there is an international consensus that such concerns should not forestall clinical development of dengue vaccines. Two essential concerns have been raised: (i) possible enhancement of the clinical response to live-attenuated dengue vaccine viruses when administered to flavivirus-immune individuals, and (ii) a sub-immunogenic vaccine, or a vaccine whose efficacy wanes over time, could leave a recipient with an immune profile that not only fails to protect, but increases the risk for experiencing severe dengue through complex immunopathological mechanisms following subsequent natural infection.[5]

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3. Components of the guidelines

The guidelines cover methodological and ethical considerations, in addition to the discussion of clinical trial phases, including bridging studies, and basic aspects of Phase IV studies. The ultimate long-term objective and challenge for any clinical trial of a tetravalent vaccine will be to demonstrate protective efficacy against each of the four dengue virus serotypes in the absence of any short, and long-term safety concerns. The basic study design will be a double-blind, randomised, vaccine or placebo-controlled trial (DB-RCT) with individuals randomised in the same community, with a focus on an age-specific cohort of individuals at high-risk of dengue as defined by clinical records. The design should not significantly reduce dengue virus transmission. Multicentre studies are proposed to cover various circulating viruses and background flavivirus immunity.

3.1. Dengue case definitions and classifications

Foremost among methodological considerations is a clear description of the dengue case definitions, for use in the clinical trial protocol. In the case of severe dengue syndromes, a number have been reported, which although sometimes common are poorly defined; and others may only partially fulfil the four laboratory and clinical criteria for DHF.[6–9] Thus strictly following all four of the WHO criteria for definition of DHF may result in omission of some severe or even fatal cases, and as a consequence WHO classifications are currently being reassessed. This will enable the development of more serviceable clinical classifications for early diagnosis, triage, and management of patients, and provide more inclusive definitions to classify all cases of dengue.

3.2. Defining the primary end point in dengue vaccine trials

All field trials require a single, primary efficacy end point used to calculate sample size and estimate vaccine efficacy.[10] The guidelines propose and support that the only practical primary end point is laboratory detection of dengue virus in a patient with at least 2 days of fever, irrespective of disease severity. Detection is defined as direct demonstration of a dengue virus by culture, antigen assay, or viral ribonucleic acid by RT-PCR. Disease features should include undifferentiated febrile illness, dengue fever, severe dengue syndromes that do not fulfil all four DHF diagnostic criteria, classic DHF and DSS.

The number of severe cases is likely to be low and the incidence of non-severe dengue cases in the community is likely to exceed the number of severe cases. Also, trial participants who are closely followed and treated before they may develop severe symptoms may further reduce the incidence of severe cases observed. Arguably, a decrease in the number of severe cases will increase the size, duration and cost of efficacy trials, which, in turn, may undermine the practicality of conducting Phase III trials. Importantly, however, the public health impact of all dengue illness is significantly larger than the impact of DHF or severe dengue alone.

In terms of practical considerations, it is known that viral diagnostic methods, especially isolation and RT-PCR assay, are more sensitive during the first 5 days of infection. The requirement for such early viral diagnosis in turn requires an active surveillance system that captures all febrile illness and avoids missing mild dengue. High rates of serologically confirmed disease have been demonstrated in a hospital-based study with enrolment criteria of $<$72 hours of fever,[11] and in a school-based study where home visits are made within a day or two of school absence.[12]

In summary, a definition of dengue as *fever of at least two days duration in a person in whom viraemia has been confirmed by virological diagnosis* offers a feasible definition for use in primary efficacy analysis of the protective effect of prospective vaccines. The protective efficacy against virologically confirmed dengue can be established as a composite of the serotypes encountered during the trial. Seroconversion is regarded as a secondary end point.

3.3. Proposed secondary efficacy end points

Secondary efficacy end points in dengue vaccine trials may be of a descriptive nature, and may not reach a level sufficient to generate statistically significant data. However, these may add value to the trial in assessing the benefit of a candidate vaccine. Such secondary end points might include:

- Severity of virologically-confirmed dengue cases
- Virologically-confirmed efficacy by age group
- Efficacy against each of the four DV types
- Efficacy before completion of full course of vaccination
- Efficacy against 'possible' or 'probable' dengue, using serology as the basis of diagnosis in patients in whom dengue virus could not be isolated.

3.4. Choice of immunological assay

It is recommended that in all dengue clinical trials immunogenicity should be measured using assays that are as close as possible to the vaccine's postulated mechanisms of protection. As such, the dengue virus antibody neutralisation test is currently considered the most relevant assay to measure immunity. However, neutralising antibodies are *not yet proven* correlates or surrogate markers of protection, and their validation may be forthcoming from clinical trials.

Guidelines have already been developed by the WHO for the plaque reduction neutralisation test (PRNT),[13] with the aim of harmonising methodologies and increasing comparability across studies, and reference virus strains and cell substrates are available from the WHO. Control sera for assay validation are currently being developed.

3.5. Selection of sites for conducting clinical trials

Potential trial sites will need to meet a number of criteria to be eligible for consideration. Primarily, the site should be endemic for one or more dengue virus types. It is considered unrealistic to expect all four virus types to be transmitted in a single season in the same geographic area, so observation for a number of years is considered necessary, depending on the local incidence and number of dengue viruses being transmitted. At least three years of background data on the epidemiology of dengue for the site should be available, which requires good community- or laboratory-based surveillance. Particularly important will be documentation of all species of flaviviruses circulating in the trial site since subclinical infections by some viruses may confound serological results and conceivably affect the course of dengue illness. On a more practical level of logistics, there must be firm commitment from the national regulatory authority (NRA), local authorities and the study population to conduct the trial and its associated investigations. In addition, the NRA should be competent to assess clinical trial protocols, and appropriate medical, community and political support needs to be assured for the clinical trial.

3.6. Safety

The following safety schedule is proposed for dengue vaccine trials:

- Pre-licensure short-term, Phases I to III
  - Monitoring should take place during days 1–21 of clinical reactions after vaccination.

- Pre-licensure long-term, Phases II and III
  - Monitoring of serious adverse reactions (SAEs) should take place for six months or more after the last vaccination, and the relative risk of dengue severity in all vaccinees compared with controls for at least 3–5 years in endemic trial sites. A Phase III trial could be stopped after 1–2 years to assess efficacy and continue for 2–4 more years to assess long-term safety, even beyond licensure.
- Post-licensure, Phase IV
  - The safety schedule should be extended to follow-up of the participants enrolled in Phase III and IV trials, and include national/regional epidemiological dengue surveillance after licensure. This approach is to identify safety signals related to rare events and extend the veracity of the conclusions drawn from the original dataset.

3.7. Additional considerations for dengue vaccine trials

The guideline emphasises the importance of first achieving licensure for any one vaccine and confirming at least one immune assay which predicts protection against dengue illness. Additional bridging studies may be necessary to extend conclusions regarding vaccine characteristics, such as protective efficacy, or immunogenicity, from one population or another, or from one manufacturing process to another.

It is likely that in children a dengue vaccine will be given in a previously defined paediatric immunisation programme. Co-administration studies to measure the safety and relative immunogenicity of the dengue vaccine itself, as well as those used in parallel, will therefore also be necessary.

Finally, post-licensure Phase IV studies will be needed to provide robust assessments of vaccine safety, particularly after use in flavivirus-immune populations and those in which other flaviviruses circulate. These will also provide estimates of the long-term effectiveness of immunisation against multiple, circulating dengue virus types in a large population, and help establish the need for booster immunisations. The decision to conduct an early, population-based Phase IV trial immediately following licensure, and/or as a condition of licensure, would lie with the NRA.

3.8. Ethical considerations

As with any new vaccine, clinical trials of dengue vaccines are subject to ethical constraints, and conformity to both the national regulations and international ethical standards outlined by WHO, ICH, and FDA guidelines for good clinical practice.[14–18]

4. The next stage of development

The current trial guidelines,[1,2] as a living document, should be critically analysed, debated and adjusted where necessary. In order to develop their relevance further, feedback from users, particularly regulators and vaccine developers, will be solicited. Further, the monitoring of emerging issues that are relevant to trials is essential, with particular emphasis on dengue case classification, diagnostics and assay systems, and trial results, before incorporation into WHO written standards.

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Conflict of Interest

The author is a staff member of the World Health Organization. The author alone is responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

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Appendix 1

These guidelines were prepared by Robert Edelman, Associate Director for Clinical Research, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA: Edelman R, Hombach J. Guidelines for the clinical evaluation of dengue vaccines in endemic areas: Summary of a World Health Organization Technical Consultation. *Vaccine* 2008;26:4113–9.

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