Letter to Editor

Commentary on the article: **Efficacy of a Tetravalent Dengue Vaccine in Healthy Children and Adolescents** (NEJM, Nov 21 2019)

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Abstract

In this Letter to the Editor, this author comments on the article in the NEJM (Nov 21 2019) in the above title on the TAK-003 Phase III trial from a point of comparing with the predecessor, the CYD-TDV, beside comparing the TAK-003 and Severe dengue, and the TAK-003 and hospitalizations. The author also queries why the vaccine must be a chimeric-vaccine, questions the sample-size in the Phase III trial and questions the Serious Adverse Events reported observed in the trial. He write on Antibody Directed Enhancement in a relation to the TAK-003. The author make Additional Observations and seek certain Explanation.

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Commentary on the article: **Efficacy of a Tetravalent Dengue Vaccine in Healthy Children and Adolescents** (NEJM, Nov 21 2019)

In the said article, the authors write on primary efficacy data from part 1 of a present Phase III randomized trial of a tetravalent dengue vaccine candidate (TAK-003) in selected countries in Asia and Latin America.

The authors conclude: 'In conclusion, TAK-003 was efficacious against virologically confirmed dengue fever among healthy children and adolescents 4 to 16 years of age, irrespective of previous dengue exposure.' [1]

At the beginning, the authors state: 'Four serotypes of dengue virus (DENV-1 through DENV-4) frequently co-circulate in areas in which the disease is endemic. Although infection provides decades of protective immunity against the infecting serotype, secondary infection with a different serotype increases the risk of severe disease.' [1 - 4]

THE CYD-TDV

'A tetravalent dengue vaccine based on a yellow fever viral "backbone," CYD-TDV (Dengvaxia, Sanofi Pasteur), has been licensed in several countries on the basis of a 56 to 61% vaccine efficacy against virologically confirmed dengue among children in Asia and Latin America. CYD-TDV is associated with an increased risk of severe dengue and dengue leading to hospitalization in seronegative persons, which has led to recommendations

that it be provided only to persons with evidence of past infection. This leaves a substantial unmet need.'[1]

THE TAK-003 AND SEVERE DENGUE

First and importantly, although here the authors define 'severe dengue' in the Supplementary Appendix to include both DHF and non-DHF severe dengue by definition, in the manuscript-text the authors list dengue haemorrhagic fever exclusive of severe dengue: 'A total of 2 cases of severe dengue (both DENV-3) and 5 cases of dengue hemorrhagic fever (3 DENV-2 and 2 DENV-3) were reported' [1].

The rate (incidence) of severe dengue is associated with factors not unrelated to host (age, phenotype, presence of comorbidities, immune-genetic profile, sequential infection), to virus-factors (serotype, strain, genotype) and to environmental aspects favoring vector proliferation [2–4].

According to the CDC, severe dengue is defined as dengue with any of the following symptoms: severe plasma leakage causing shock or fluid accumulation leading to respiratory distress; severe bleeding; or severe organ involvement such as increased transaminases ≥1,000 IU/L, impaired consciousness, or heart ailment [5].

From 1975 to 2009, symptomatic dengue virus infections had been classified according to the WHO guidelines as dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS, the most severe form of DHF). In 2009, the case definition became changed to the new clinical classification after feedback that the case definition of DHF is found both very difficult to apply in resource-limited settings and too specific, in the reason the definition failed to identify a substantial proportion of severe dengue cases, including cases of hepatic failure and

encephalitis. The 2009 clinical classification became criticized as overly inclusive, in the reason it allows many a different way to qualify as severe dengue, and nonspecific warning signs are used as diagnostic criteria in dengue. Finally, the new guidelines have been criticized in the reason they do not define the clinical criteria in establishing severe dengue (with the exception of providing laboratory cutoff values of transaminase levels), thus leaving severity determination to individual clinical judgment.'[5]

The WHO still seem not to describe severe dengue as more than the classical DHF and DSS: Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment [6] .

The authors state: 'The severity of virologically confirmed dengue is assessed with the use of two approaches: blinded review by the dengue case adjudication committee (DCAC) , using predefined criteria, and with a program for analyzing data in accordance with World Health Organization (WHO) 1997 criteria for dengue hemorrhagic fever.' [1], but only describe the first of these two approaches as in the Table 1 here [1]. Again, it is seen here that the DCAC do not separate DHF from severe dengue in the reason that the Group 1 and Group 2 in the Table 1 describe DHF.

Table 1. The DCAC severe dengue case-criteria applied in a blinded-manner to virologically-confirmed hospitalized cases [1]

Organ/Function	Observation
Bleeding abnormality	Requiring significant intervention such as blood transfusion, nasal packing, hormonal therapy or bleeding in critical organs
Plasma leakage	Accompanied by functional impairment such as shock and respiratory distress by clinical evidence, radiological evidence or hematocrit elevated > 20%
Hepatitis and functional liver-impairment	Increased AST and PT. Hypoalbuminemia.
Renal impairment	Requiring dialysis
Cardiac abnormality	Intrinsic, not due to volume-depletion e.g. myocarditis, pericarditis, myo-pericarditis and new conduction abnormality
CNS involvement	Except simple febrile fit or brief delirium
Shock	All shock cases

DCAC Members were not study-investigators reviewed the total data [1].

HOSPITALIZATION

Secondly, presuming the hospitalizations are due to dengue, not all cases of dengue fever are hospitalized – generally, cases of mild dengue are not hospitalized and managed on an outpatient-basis with follow-up. It is reasonable to presume then that hospitalizations in the context of this vaccine-trial are in the reason of severe dengue, but there is need in the authors to state so and list the reasons that the subjects became hospitalized – severity of illness and cost of treatment remaining in a concern here.

'All non-hospitalized cases were considered non-severe by the sponsor' [1].

'The efficacy of the vaccine against dengue leading to hospitalization is encouraging. However, this finding may be confounded by the high

proportion of cases of dengue that led to hospitalization in which DENV-2 was the causal serotype (43 of 58 overall cases leading to hospitalization in the analysis of the primary end point), mainly in Sri Lanka.' [1] This Finding, hospitalizations are very much additionally frequent in Sri Lanka compared to the remaining countries, require further thought and explanation given.

Thirdly, the authors not seem to have learnt from the experience of the CYD-TDV and entirely fail to talk about Antibody Directed Enhancement (ADE), the reason that the 'CYD-TDV is associated with an increased risk of severe dengue and dengue leading to hospitalization in seronegative persons' 1 as stated early by the authors and that 'secondary infection with a different serotype increases the risk of severe disease' [1], as also relevantly stated early by the authors.

The increased number of severe dengue among those vaccinated with the CYD-TDV in a very early mass-vaccination programme among children in the Philippines had been acknowledged on the follow-up by the Dengvaxia manufacture, Sanofi [2, 3, 7 – 9]

WHY CHIMERIC VACCINE?

Next, in observing the pattern of the degree of effectiveness of this TAK-003 the question arises why the vaccine could not have been made entirely out of live-attenuated viruses of the four strains instead of chimera in the case of DENV1, DENV3, and DENV4: 'The vaccine had 97.7% efficacy against DENV-2, 73.7% efficacy against DENV-1, and 62.6% efficacy against DENV-3; however, the results for efficacy against DENV-4 were inconclusive (63.2%; 95% CI, −64.6 to 91.8)'

Stinchcomb D of Inviragen, who is responsible in the initial development work of the TAK-003, writing in his patent 'Compositions and methods for rapid immunization against dengue virus' (June 2017) state 'embodiments disclosed herein relate to methods and compositions for inducing protection in a subject against all dengue virus serotypes by, for example, administering a vaccine to a subject against all dengue virus serotypes in two or more doses on one or more than one anatomical location consecutively within a short interval of time. Some embodiments can include introducing a vaccine composition to a subject via intradermal (ID), subcutaneous (SC), or intramuscular (IM) injection in one location and consecutively in another anatomical location by ID, SC, IM or by other

introduction method at a second different anatomical location. Additional embodiments include using any combination of modes of administration for introducing a dengue virus vaccine of all dengue virus serotypes to a subject where administration of the vaccine occurs at two or more anatomical sites or by two or more different routes consecutively on the same day to the subject.' [10]

SAMPLE SIZE

Next, the authors here state: 'The sample size calculation was based on the assumption of a true vaccine efficacy of 60% and a background annual dengue incidence of 1%. We calculated that a sample of 20,100 participants undergoing randomization in a 2:1 ratio (TAK-003: placebo) would enable identification of 120 cases of virologically confirmed dengue from 30 days after the second vaccination to the end of part 1, providing at least 90% power to rule out a vaccine efficacy of 25% or less (with a two-sided significance level of 0.05). 27.7% of participants were seronegative at baseline' [1]

The authors state: 'The highest percentage of seronegative participants was in Panama (62.2%), followed by Sri Lanka (38.5%), Thailand (34.4%), Brazil (28.8%), Nicaragua (22.3%), Colombia (15.4%), the Philippines (12.4%), and the Dominican Republic (2.8%).' [1] In view of the wide variation in the percentage seronegative, the authors do need to disclose the predominant DENV-strain and the prevalence-pattern of these strain.

In any region or country, the various sero-types gradually begin to predominate over the remaining sero-types over the years. The predominant sero-type differs according to various geography, country, region, season and over time [2 - 3]

Next, the authors state: 'Exploratory analysis of the secondary efficacy end points showed that the vaccine had 97.7% efficacy against DENV-2, 73.7% efficacy against DENV-1, and 62.6% efficacy against DENV-3; however, the results for efficacy against DENV-4 were inconclusive (63.2%; 95% CI, −64.6 to 91.8).' [1] The authors need to attempt to explain the wide-variation in the efficacy, especially that the vaccine is ineffective against DENV4.

'The number of cases identified was sufficient to provide estimates of vaccine efficacy against three of the serotypes but not against DENV-4.' [1]

The researchers could have anticipated this and increased the sample-size at the start. The dengue-rate (incidence) by strain in each of these countries could have offered a guide.

‘The distribution of severe dengue and dengue hemorrhagic fever remained favorable for the vaccine, although the small number of cases limits any meaningful conclusion.’ [1] Again, the researchers could have predicted this, and could have increased the sample-size at the start.

The statement ‘No cases were reported in Nicaragua or the Dominican Republic’ [1] is also found disturbing. The researchers must have selected the participating countries based on the rate (incidence) of dengue in these countries.

EXPLANATIONS REQUIRED

‘Overall, efficacy was broadly similar across age ranges (72.8% to 83.3%) and among participants who were seronegative at baseline (74.9%) and those who were seropositive at baseline (82.2%)’ [1]

‘The vaccine efficacy against DENV-1 was 79.8% among participants who were seropositive at baseline (95% CI, 51.3 to 91.6; 7 cases in the vaccine group vs. 17 in the placebo group) and was 67.2% among those who were seronegative at baseline (95% CI, 23.2 to 86.0; 9 cases in the vaccine group vs. 13 in the placebo group); against DENV-2, the corresponding vaccine efficacy values were 96.5% (95% CI, 88.7 to 98.8; 3 cases vs. 42 cases) and 100% (0 cases vs. 22 cases).’ [1] An explanation should be sought that in DENV-2, the vaccine is of greater efficacy in the seronegatives instead of with the seropositives.

‘The results for DENV-3 among participants who were seronegative at baseline were inconclusive but did not suggest efficacy (–38.7%; 95% CI, –335.7 to 55.8; 11 cases in the vaccine group vs. 4 cases in the placebo group), whereas the efficacy among participants who were seropositive at baseline was 71.3% (95% CI, 54.2 to 82.0; 28 cases in the vaccine group vs. 47 in the placebo group).’ [1] This paradox seen against DENV-3 in comparison with DENV-1 and DENV-2 must be explained.

‘No cases of virologically confirmed dengue caused by DENV-4 were observed among participants who were seronegative at baseline.’ [1] Observing that the vaccine is overall ineffective against DENV4, it is

reasonable conclusion that it is also ineffective in seronegatives against DENV4.

Table 1 of the article indicates the efficacy of the vaccine by variables, but it is quite surprising that the vaccine is totally ineffective against severe dengue and DHF (both by the authors' definition) when found efficacious to various extent except against DENV4. The authors must attempt to explain this observation.

SERIOUS ADVERSE EVENTS

'One vaccine recipient and four placebo recipients had serious adverse events that were considered by the investigator to be related to vaccine or placebo (two had hypersensitivity, two received a diagnosis of dengue, and one had dengue hemorrhagic fever)' [1]. The authors do need to explain the reason the authors include dengue and DHF as 'adverse events'.

'The most commonly reported unsolicited adverse events (reported by ≥1% of vaccine recipients) within 4 weeks after any dose were nasopharyngitis (2.7% in the vaccine group and 3.0% in the placebo group), upper respiratory tract infection (2.6% and 2.9%, respectively), and viral infection (1.1% and 0.9%).' [1] Are these events mutually exclusive in terminology? Why do the authors think of infections unrelated to dengue as Adverse Events?

ANTIBODY DIRECTED ENHANCEMENT

It had previously been pointed out on the CYD-TDV that in finding a dengue vaccine to be really worth it, the efficacy of the vaccine to each of the strain must be above 90%, particularly among seronegatives. If not, the vaccine primes the receiver and causes ADE leading to DHF when the receiver subsequently becomes infected by natural-infection with a strain that the vaccine does not adequately protect against [2 – 4, 11].

ADE has been proven to happen both here *in vitro* and *in vivo*.

Antibody-dependent enhancement (ADE) happen when non-neutralizing antiviral protein facilitate virus entry in the host-cell, leading to increased infectivity of the cell. Some cell are not found to have the usual receptor(s) on the surface that viruses use to find access. The antiviral protein(s) (i.e., the antibodies) bind to antibody Fc receptor that some of these cell have in

the plasma-membrane. The viruses bind to the antigen binding-site at the opposite end of the antibody. ADE is frequently seen in cell cultured in the laboratory, but rarely happen *in vivo* except in dengue virus. The dengue virus uses such mechanism to infect the human-macrophage, rendering a usually mild viral infection to become life-threatening [12].

The most widely known example of ADE happen in the situation of infection with dengue virus [13].

The phenomenon of ADE can be observed when people who had previously been infected with one serotype of dengue virus becomes infected months, or years, after with a different serotype. In such cases, the clinical manifestation of the disease is additionally severe, and these people have increased viremia compared with those in whom ADE is not seen. Such explain the observation here, whereas primary (first) infections cause mostly mild disease (dengue fever), secondary infection (re-infection at a subsequent date) is found in added likely to be associated with DHF and DSS in ~~both~~ children and adults [14].

Infection with dengue virus ~~induce~~ cause the production of neutralizing homotypic immunoglobulin G (IgG) antibodies that provide immunity throughout life against the specific sero-type. Infection ~~with dengue virus~~ also produces some amount of cross-protective immunity against the remaining three sero-types [15].

Neutralizing heterotypic (cross-reactive) IgG antibodies are responsible in this cross-protective immunity, which normally persist a period of a few month to a few year. These heterotypic antibody titers decrease over some lengthy period (4 to 20 years) [15]. While heterotypic IgG antibody titers decrease, homotypic IgG antibody titers increase over such period. This is due to the preferential survival of lengthy-lived memory B-cells producing homotypic-antibodies [15].

~~In addition to~~ Beside inducing neutralizing heterotypic antibodies, dengue-infection could also induce heterotypic antibodies that neutralize the virus only partially or not at all [16]. The production of such cross-reactive but non-neutralizing antibodies could be the reason in severe secondary infection. It is thought here that by binding to but not neutralizing the virus, such antibodies cause it to behave as a 'trojan horse' [17 -18] when such is delivered the wrong part of dendritic-cells that have ingested the virus

targeted to destruction [19 – 20] When inside the white blood-cell, the virus multiply undetected, eventually generating very high virus-titers which cause severe disease [21].

A study conducted tried to explain how non-neutralizing antibodies down regulate the immune response in the host cell identified as a major basis in cytokines production. In vitro experiments show that the inflammatory cytokines and type 1 interferon production became reduced when the ADE-dengue virus complex bound to the Fc receptor of THP-1 cells.

As an example of ADE happening *in vivo*: an epidemic of dengue fever of the DENV-1 sero-type happened in Cuba from 1977 to 1979. ~~The infecting serotype was DENV-1~~. ~~The epidemic was followed by~~ Subsequently, two additional outbreaks happened - one in 1981 and one in 1997, in both DENV-2 became the infecting serotype. 205 cases of DHF and DSS happened during the 1997 outbreak. All but three of these cases were demonstrated to have been previously infected by the DENV-1 serotype during the epidemic of 1977–1979 [22].

Additionally, those ~~who had been~~ infected with DENV-1 during the 1977-79 outbreak and subsequently secondarily infected with DENV-2 in 1997 had a 3 - 4 fold increased probability of having severe dengue than those secondarily infected with DENV-2 in 1981 [15]. Such a scenario is explained by the presence of neutralizing heterotypic IgG antibodies in enough titers in 1981, the titers of which had decreased by 1997 to the concentration where they not any longer provided significant cross-protective immunity.

In ADE of a viral infection the body releases cytokines that cause the endothelial tissue to become permeable which results in DHF and fluid loss from the blood vessels [23].

ADDITIONAL OBSERVATION

Table 1 of the article, Baseline seropositivity rates (number and % of evaluated participants) by dengue serotype and combinations, remains incomprehensible in view of the numbers shown.

There is also need here that the TAK-003 authors reveal the details of efficacies in each country that the Phase III had been carried out. Details on the CYD-TDV showed that the vaccine was a total failure in Puerto Rico and Mexico [11].

CONCLUSION

In 2017, the TAK-003 manufacturer Takeda revealed the finding on the Phase 2 trial: 'In participants who were sero-negative at baseline, a second-dose given at Month 3 improved the tetravalent sero-positivity rate at Month 6 to 86%, compared to 69% in the one-dose group. A booster dose at Month 12 resulted in a 100% tetravalent sero-positivity rate at Month 13 in participants who were sero-negative at baseline' [2 – 3, 24] .

Thus, the results of this Phase III trial is quite unexpected.

There is a need to give thought here to carry out the Phase III trial again. And, there is hope also with the US NIH TV003/005 presently on Phase III trial [25 - 27]

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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