Efficacy of tetravalent dengue vaccine in Thai schoolchildren

The paper by Arunee Sabchareon and colleagues (Nov 3, p 1559)[1] reports the results of a trial of a promising dengue vaccine in an endemic area of Thailand. I have some issues with the table of baseline characteristics (table 1), which compares the mean ages, with SDs, of 4000 children (efficacy analysis) with a convenience subsample of 300 first enrollees (immunogenicity).

First, medians and ranges—juxtaposed with means and SDs—of the two main groups (preallocation of treatments) might better quantify the possibility of sampling bias for group-level continuous variables (age). Second, Sabchareon and colleagues, and Scott Halstead in his accompanying Comment,[2] do not clearly explain the need for random selection of immunogenicity subsamples ("pre-bled random sample").[2] A random sample related to random allocation of vaccine or placebo does not mean absence of sampling bias, addressed through random selection of subsamples.

The implications are that: (1) high awareness of dengue infections and parental fears could combine to skew subsamples of only the earliest to be enrolled towards younger children; and (2) age-based sampling bias (immunogenicity) could contribute to disappointment in the efficacy of dengue vaccines in paediatric trials, particularly for the most prevalent of the types (DENV2) during this trial.[3,4]

More clarity is needed within CONSORT's minimum standards for depicting tabular comparisons of baseline characteristics between two groups randomised to treatment or placebo.[5] It is inappropriate and misleading to include data from non-randomly-selected subpopulations of the main treatment and placebo groups, when the standards are based on an inappropriate assumption that differences are due to chance alone.

My views should not be construed as policies or views of the US Food and Drug Administration. I declare that I have no conflicts of interest.

Linda McKibben
linda.mckibben@fda.hhs.gov

Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD 20901, USA

1 Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 2012; **380:** 1559–67.
2 Halstead SB. Dengue vaccine development: a 75% solution? *Lancet* 2012; **380:** 1535–36.
3 WHO. Guidelines for the clinical evaluation of dengue vaccines in endemic areas. http://whqlibdoc.who.int/hq/2008/WHO_IVB_08.12_eng.pdf (accessed March 13, 2013).
4 Lanata CF, Andrade T, Gil AI, et al. Immunogenicity and safety of tetravalent dengue vaccine in 2–11 year-olds previously vaccinated against yellow fever: randomized, controlled, phase II study in Piura, Peru. *Vaccine* 2012; **30:** 5935–94.
5 CONSORT. Item 15—a table showing baseline demographic and clinical characteristics for each group. http://www.consort-statement.org/consort-statement/13-19---results/item15_baseline-data/ (accessed March 13, 2013).

There has been much interest in a safe dengue vaccine with the potential to induce protection against all four dengue virus (DENV) serotypes. We are perplexed by the results of Arunee Sabchareon and colleagues' study,[1] which shows that, despite the induction of reasonable levels of neutralising antibodies against all four serotypes on vaccination (73–100% seropositivity), protection against DENV2 infection was minimal (efficacy of 9·2% after three vaccinations).

Sabchareon and colleagues assessed neutralising antibody titres with the plaque reduction neutralisation test (PRNT).[1,2] This assay is regarded as the most reliable way to measure neutralising antibodies, and the test usually uses Fcγ-receptor-negative (FcγR-negative) cells. A group of major target cells of DENV in vivo are, however, FcγR-bearing monocyte-lineage cells. Sabchareon and colleagues question the PRNT as a means to reflect protection in vivo and suggest that a system modelled on in-vivo target cells merits further study. We have found that the neutralising activities of some serum samples, as determined by PRNT assays that use FcγR-negative cells, were absent or at much lower concentrations than when measured with FcγR-positive cells.[3,4] Our study suggests the presence of DENV antibody complexes that are incapable of infecting FcγR-negative cells, but retain infectivity for FcγR-expressing cells.[5]

The breakthrough by DENV2 infection could be caused by the dominance of antibodies that might not confer virus neutralisation in the presence of FcγR. We therefore advocate for further efforts to investigate vaccine efficacy through in-vitro assays based on the knowledge of DENV target cells in vivo—ie, use of FcγR-bearing cells in PRNT assays.

We declare that we have no conflicts of interest.

*Meng Ling Moi, Tomohiko Takasaki, *Ichiro Kurane*
kurane@nih.go.jp

National Institute of Infectious Diseases, 162-8640 Tokyo, Japan

1 Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 2012; **380:** 1559–67.
2 Guy B, Barrere B, Malinowski C, Saville M, Tessyou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent vaccine. *Vaccine* 2011; **29:** 7229–41.
3 Moi ML, Lim CK, Kotaki A, Takasaki T, Kurane I. Discrepancy in neutralizing antibody titers between plaque reduction neutralizing tests using FcgammaR-negative and FcgammaR-expressing BHK-21 cells. *Clin Vac Immunol* 2010; **17:** 402–07.
4 Moi ML, Lim CK, Chua KB, Takasaki T, Kurane I. Dengue virus infection-enhancing activity in serum samples with neutralizing activity as determined by using FcγR-expressing cells. *PLoS Negl Trop Dis* 2012; **6:** e1536.
5 Moi ML, Lim CK, Kotaki A, Takasaki T, Kurane I. Detection of higher levels of dengue viremia using FcγR-expressing BHK-21 cells than FcγR negative cells in secondary infection but not in primary infection. *J Infect Dis* 2011; **203:** 1405–14.

Authors' reply

We appreciate the opportunity to respond to these letters on our report of the first clinical efficacy study of a dengue vaccine.[1] The main objective of this phase 2b study was to assess vaccine efficacy in 4002 children. Immunogenicity was a secondary

endpoint and assessed in a subset of 300 children. The size of this immunogenicity subset was arbitrary and was composed of the first participants enrolled in the study, as acknowledged in the paper and remarked on by Linda McKibben. The rationale was simply to facilitate the conduct and logistics of this large single-centre study. Knowing that any potential link between immune response and vaccine efficacy would be assessed with a blood sample taken after dose three in all participants, we judged that this method for the selection of the small subset was sufficient for a phase 2b study.

Table 1 of the paper compares the demographic characteristics of participants in the dengue vaccine group versus the control group but makes no comparison between the demographics of the immunological subset and the per-protocol set for efficacy (PPSE). We do not claim that the non-random nature of enrolment into the immunological subset is guaranteed free of bias; however, there is no evidence for McKibben's suggestion that the subset could be biased towards the enrolment of younger children. Age was similar between the PPSE and the immunological subset, as assessed by mean, median, SD, and range. Furthermore, the observed immune response was comparable to previously generated immunogenicity data in other phase 1 and 2 studies.[2,3]

Meng Ling Moi and colleagues advocate further investigation of in-vitro assays based on the in-vivo targets of dengue. As mentioned in our Discussion, we agree that measurement of dengue virus neutralisation in a system modelled on in-vivo targets deserves further study, and such work is ongoing.

AS declares that she has no conflicts of interest. DW, JL, AB, and AM are employees of Sanofi Pasteur and own shares or stock options in the company.

*Arunee Sabchareon, *Derek Wallace, Jean Lang, Alain Bouckenooghe, Annick Moureau*
derek.wallace@sanofipasteur.com

Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (AS); Asia-Pacific Clinical Development, Sanofi Pasteur, Singapore (DW, AB); and Research and Development, Sanofi Pasteur, Marcy l'Etoile, France (JL, AM)

1 Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet* 2012; **380:** 1559–67.
2 Capeding RZ, Luna IA, Bomasang E, et al. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in the Philippines. *Vaccine* 2011; **29:** 3863–72.
3 Tran NH, Nguyen TKT, Forrat R, Saville M, Bouckenooghe A, Wartel-Tram A. Safety and immunogenicity of recombinant, live attenuated tetravalent dengue vaccine (CYD-TDV) in healthy Vietnamese adults and children. *J Vaccines Vaccin* 2012; **3:** 162.

Evaluation of the Affordable Medicines Facility—malaria

Sarah Tougher and colleagues (Dec 1, p 1916)[1] provide an analysis of the effects of the pilot phase of the Affordable Medicines Facility—malaria (AMFm) in seven countries of sub-Saharan Africa. The paper reports increases in the availability and market share of artemisinin-based combination therapies (ACTs) and price reductions in most countries, and concludes that the AMFm can be effective in rapidly improving these outcomes.

Although we acknowledge that classic randomised study designs for assessing the effectiveness of complex interventions are not always feasible, efforts have to be made to reduce the likelihood of bias by combining different methods and data sources.[2] We are surprised that the evaluation of such a large-scale and important experiment—the pilot countries are home to a quarter of the world's malaria cases, and the cost of implementation to date is several hundred million US dollars—is based on a pre/post intervention design only, with no control countries.[3] Most of the pilot countries have major malaria initiatives in place that might have contributed to the noted effects. Since the evaluation of the AMFm relied on methods used in the ACTwatch project, a more systematic comparison with the three ACTwatch countries that did not participate in the AMFm pilot phase (Benin, Democratic Republic of Congo, and Zambia) could have been helpful. In Burkina Faso—a non-AMFm country—we have documented a rapid increase in ACT access in recent years.[4]

For the **ACTwatch project** see http://www.actwatch.info/about/

More discussion on alternatives for increasing access to quality-controlled ACTs in sub-Saharan Africa is needed, and in particular the importance of strengthening the weak health services in sub-Saharan Africa should not be overlooked.[5]

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*Yesim Tozan, Albrecht Jahn, *Olaf Müller*
olaf.mueller@urz.uni-heidelberg.de

New York University, New York, NY, USA (YT); and Ruprecht-Karls-University, Institute of Public Health, INF 324, 69120 Heidelberg, Germany (YT, AJ, OM)

1 Tougher S, Ye Y, Amuasi JA, et al, for the ACTwatch Group. Effect of the Affordable Medicines Facility—malaria (AMFm) on the availability, price, and market share of quality-assured artemisinin-based combination therapies in seven countries: a before-and-after analysis of outlet survey data. *Lancet* 2012; **380:** 1916–26.
2 Craig P, Dieppe P, Macintyre S, Michie S, Nazareth I, Petticrew M. Developing and evaluating complex interventions: the new Medical Research Council guidance. *BMJ* 2008; **337:** a1655.
3 Global Fund to Fight AIDS, Tuberculosis and Malaria. Affordable Medicines Facility—malaria (phase I): monitoring and evaluation technical framework. http://www.theglobalfund.org/documents/amfm/AMFm_Phase1MonitoringAndEvaluationTechnical_Framework_en/ (accessed Dec 14, 2012)
4 De Allegri M, Louis V, Tiendrébeogo J, et al. Moving towards universal coverage with malaria control interventions: achievements and challenges in rural Burkina Faso. *Int J Health Plann Manage* 2013; **28:** 102–21.
5 Müller O. Malaria in Africa: challenges to control and elimination in the 21st century. Frankfurt: Peter Lang Verlag, 2011.


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Authors' reply

Yesim Tozan and colleagues express concern over the pre/post study design used in the independent evaluation of the Affordable Medicines Facility—malaria (AMFm), and suggest that the use of comparator countries would