Report of the Meeting of the WHO Advisory Committee on Dengue and other Flavivirus Vaccines

13-14 May 2009
WHO-HQ, Geneva, Switzerland
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Acknowledgments

Dr Anna Durbin, Johns Hopkins University, kindly served as rapporteur to this meeting.
# Abbreviations and Acronyms

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<th>Abbreviation</th>
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<tbody>
<tr>
<td>CMI</td>
<td>cell-mediated immunity</td>
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<tr>
<td>DF</td>
<td>dengue fever</td>
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<td>DHF</td>
<td>dengue haemorrhagic fever</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DSS</td>
<td>dengue shock syndrome</td>
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<td>FDA</td>
<td>Food and Drug Administration (USA)</td>
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<td>E</td>
<td>envelope protein</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EU</td>
<td>European Union</td>
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<tr>
<td>GAVI</td>
<td>Global Alliance for Vaccines and Immunization</td>
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<tr>
<td>cGMP</td>
<td>current good manufacturing practice</td>
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<td>GMT</td>
<td>geometric mean titre</td>
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<td>GSK</td>
<td>GlaxoSmithKline</td>
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<tr>
<td>Ig</td>
<td>immunoglobulin</td>
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<tr>
<td>IVR</td>
<td>Initiative for Vaccine Research (WHO)</td>
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<td>JE</td>
<td>Japanese encephalitis</td>
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<td>JE-CV</td>
<td>live attenuated chimeric JE vaccine</td>
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<td>JEV</td>
<td>Japanese encephalitis vaccine</td>
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<td>LJEV</td>
<td>live Japanese encephalitis vaccine</td>
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<td>MBDV</td>
<td>mouse brain-derived vaccine</td>
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<td>MV</td>
<td>measles vaccine</td>
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<tr>
<td>NS1</td>
<td>nonstructural protein 1</td>
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<tr>
<td>PATH</td>
<td>Program for Appropriate Technology in Health</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PFU</td>
<td>plaque-forming unit</td>
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<td>PrM</td>
<td>pre-membrane protein</td>
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<tr>
<td>PRNT</td>
<td>plaque reduction neutralization test</td>
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<tr>
<td>R&amp;D</td>
<td>research &amp; development</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>SAGE</td>
<td>Strategic Advisory Group of Experts (WHO)</td>
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<td>SAE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>TBE</td>
<td>tick-borne encephalitis</td>
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<tr>
<td>TDR</td>
<td>Special Programme for Research and Training in Tropical Diseases</td>
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<td>VJE</td>
<td>vero cell-produced Japanese encephalitis vaccine</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>YF</td>
<td>yellow fever</td>
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Introduction

The Advisory Committee on Dengue and other Flavivirus Vaccines provides technical and strategic advice to the WHO Initiative for Vaccine Research (IVR). The Committee advises on issues related to dengue and other flaviviruses vaccine research and development (R&D), particularly on those issues related to the facilitation of and coordination with the international dengue and other flaviviruses vaccine R&D effort, and with a special emphasis on public-health needs of developing countries, as well as on the development of guidance on preclinical and clinical evaluation of dengue and other flaviviruses vaccines to support the development of norms and standards for those vaccines.

The 2009 meeting included consultations with commercial developers of late-stage vaccine candidates against dengue and Japanese encephalitis. These sessions were confidential, and the respective sections in this report have been cleared by the companies for public release.
1. JE control efforts in the region and vaccine needs

The Committee was informed by Joachim Hombach (WHO) on the status of Japanese encephalitis (JE) control efforts. Basis for the control efforts are the WHO position paper on JE immunization that was last updated in 2006. A further revision might be envisaged once more data have been assembled on disease and immunization strategies, and if the vaccine landscape changes. Limitations to the current knowledge of JE epidemiology in endemic regions were acknowledged. The Strategic Advisory Group of Experts (SAGE) has already called for a better assessment of disease burden and efforts to identify the target population for immunization with a JE vaccine. In addition, a review of the regional JE control goals should be performed. The lack of a WHO prequalified vaccine limits recommendations the WHO can make in relation to specific vaccines.

JE disease surveillance is critical, and substantive work has been done in the South-East Asia and Western Pacific regions. Surveillance efforts have intensified in several countries, including Cambodia, China, India, Indonesia, Nepal, the Philippines and Viet Nam. However, laboratory support for virologic and serologic confirmation of JE must be further enhanced and reporting must be improved.

Three vaccines are likely to be submitted for WHO prequalification over the coming years: the live attenuated SA 14-14-2 vaccine; the inactivated cell-based SA 14-14-2 vaccine and a live, recombinant JE vaccine. No information is currently available on a future public-sector price for the latter two vaccines.

In relation to the most widely-used product, the live attenuated SA 14-14-2 vaccine, the following data needs were highlighted:

- Long-term vaccine efficacy and booster needs.
- What is the relevance of the minor drop in measles geometric mean titre (GMT) seen when the vaccine is co-administered with measles vaccine?
- Documentation of the long-term safety of the vaccine.

Another concern raised was funding for a JE vaccine effort. The PATH JE programme is ending its funding stream and, thus far, no other entity has stepped in to take on this process for new and underutilized vaccines. It is expected that there will be a prequalified JE vaccine in the next 3+ years and that GAVI funding will be available at that time for vaccine purchase, at least for catch-up campaigns. The WHO Secretariat, with technical partners, will assist in the advocacy, fundraising, coordination and implementation of a bi-regional control strategy. The fourth bi-regional meeting on JE was scheduled to take place in Bangkok, Thailand on 8–9 June 2009.
Finally, it was noted that the WHO written standard on live attenuated JE vaccines (WHO Technical Report Series No. 910, 2002) should be updated to include the live attenuated chimeric JE vaccine JE-CV.
2. Inactivated JE vaccine candidate IXIARO®, Intercell

IXIARO® is an inactivated JE vaccine using the SA 14-14-2 strain, and is produced by Intercell. It was approved by the FDA on 30 March 2009, by the European Medicines Agency on 31 March 2009, by the Therapeutic Goods Agency in Australia (licence-holder CSL, trade name JESPECT®) on 30 January 2009 and by Health Canada on 30 October 2009, as a traveller’s vaccine for the prevention of Japanese encephalitis, and for use by the military for personnel deployed to affected regions of the world. Intercell Biomedical Ltd. in Scotland is the manufacturer of vaccine which is currently distributed in Australia, Canada, the European Union (EU) and the United States of America (USA). In the future, it is expected to be licensed in China, Hong Kong Special Administrative Region, Singapore and some countries in Latin America. Biological E of India will be the licence holder and manufacturer for most countries in Asia. Intercell is the licence holder for vaccine in Canada, the EU and the USA, and CSL is the licence holder in Australia. The vaccine comes in a liquid, preservative- and stabilizer-free presentation using prefilled syringes.

IXIARO® was initially developed by the Walter Reed Army Institute of Research. Intercell has conducted 10 trials in adults of the candidate vaccine under the name IC51, one of which is still ongoing. More than 4146 adults and 48 children have received the IC51 vaccine under the clinical trials. For these trials, seroconversion was defined as a plaque-reduction neutralization titre 50% (PRNT$_{50}$) of $\geq 1:10$ [1, 2]. Intercell validated and standardized their plaque reduction neutralization test (PRNT) assay across studies [3] and it was agreed by various regulatory authorities that use of the PRNT$_{50}$ as a correlate of protection was an acceptable approach.

The immunogenicity of IC51, as determined in two Phase III trials, was described. IC51 given at day 0 and day 28 was compared with JE-VAX® given at days 0, 7, and 28. PRNT50 results were comparable for the two vaccines. Antibodies were broadly neutralizing against a panel of JE viruses from all four genotypes and were comparable to those induced by JE-VAX®. No safety issues were identified during the 24 month long-term follow-up period included in one study. The vaccine produced protective antibody titres ($\geq 1:10$) in 98.9% of vaccinees at two months, 95% at six months, 83.4% at 12 months and 81.8% at 24 months.

In a randomized, observer blinded, parallel group Phase III study designed to study the immune kinetics and single-dose schedule of IC51, 374 healthy adults were randomized to receive two doses of 6 mg at days 0 and 28 (125 subjects), a single dose of 12 µg at day 0 (124 subjects), or a single dose of 6 µg at day 0 (125 subjects). The day 0/28 schedule provided protective antibody levels in 97.3% of subjects from one week post-vaccination out to study day 56, with peak PRNT$_{50}$ occurring at study day 35 post first vaccination. The single 12 µg dose provided protective antibody titres in 66% of subjects.
The single 6 µg dose provided protective antibody titres in 43% and 26% of subjects at study day 28 and 56, respectively. The long-term protection and immunogenicity of a booster dose of IC51 was evaluated in an open-label Phase III follow-on trial to the above study. The primary endpoint of the trial was the seroprotection rate at month 24. Those subjects with a PRNT$_{50}$ < 1:10 at month six received a boost at 11 months. Those subjects with a PRNT$_{50}$ < 1:10 at month 12 received a booster at month 23. Eighty-three percent of subjects who received two doses of 6 µg were protected at six months post-vaccination compared with 15% of subjects who received a single 12 µg of IC51 and 9% of those who received a single 6 µg dose. At 12 months post-vaccination protection rates declined to 58%, 8%, and 4% for each respective group. At 24 months post vaccination, protection rates were 48%, 6%, and 4% for each respective group. For these analyses subjects who received booster doses during the study were counted as non-protected. Sixteen subjects who received two doses of 6 µg of IC51, 84 who received a single 12 µg dose, and 96 who received a single 6 µg dose received a booster dose at 11 months. Ninety-nine to 100% of subjects who received a booster dose at 11 months seroconverted. Twenty-four subjects in the 2 x 6 µg group, 12 subjects in the single 12 µg group, and three subjects in the single 6 µg group received a booster dose of IC51 at month 23; 100% seroconverted. Forty-eight percent of subjects in the IC51-305 trial vaccinated with two 6 µg doses of IC51 had protective antibody titres at month 24 compared with 82% of subjects in the IC51-303 trial, given the same dose and schedule. Vaccine-related issues, such as batch difference and batch age, could be excluded as causes for the difference. The only prominent difference in baseline characteristics of study populations was the tick-borne encephalitis (TBE) vaccination rate. Approximately 75% of subjects enrolled in IC51-303 had pre-existing immunity to TBE compared with 0% of subjects enrolled in IC51-305. Random variation between trials can also not be excluded as a reason for the observed difference.

Co-administration of IXIARO® with hepatitis A vaccine (HAVRIX®) was evaluated in 192 healthy adult subjects. There was essentially no difference in the GMT for Japanese encephalitis vaccine (JEV) neutralizing antibody in subjects who received IXIARO® alone compared with those who received IXIARO® with HAVRIX® with 98% and 100% seroconverting to JEV, respectively. In addition, there was essentially no difference in seroconversion rates to HAVRIX® between groups with 96% of those receiving HAVRIX® alone seroconverting compared with 100% of those who received IXIARO® with HAVRIX®.

The safety database for IXIARO® is compiled from studies across the programme. The safety profile of IXIARO® was similar to placebo with approximately 40% of subjects in both groups experiencing at least one vaccine-related adverse event following immunization. Local tolerability of IXIARO® was compared to JE-VAX®; 2.1% (9/421) of subjects who received IXIARO® reported at least one severe local reaction compared with 13.8% (59/427) of subjects who received JE-VAX® (p < 0.0001). No cases of angioedema, anaphylaxis, bronchospasm, encephalitis, meningitis, or acute disseminating encephalomyelitis have occurred with IXIARO®.
IC51 has been evaluated in 48 children as part of the IXIARO® clinical programme in children for travel indication. In an open-label, randomized, paediatric Phase II dose confirmation study conducted in India in healthy children ≥ 1 year – < 3 years of age, children were randomized 2:2:1 to receive IC51 0.25 mL (3 µg) intramuscularly on days 0 and 28 (24 subjects), 0.5 mL (6 µg) intramuscularly IC51 on days 0 and 28 (24 subjects), or JenceVac® 0.5 mL subcutaneously on days 0, 7, and 28 (12 subjects). Seroconversion rates at day 56 to IC51, when administered as two doses of 3 µg or two doses of 6 µg, were comparable, and compared favourably with seroconversion rates to three doses of JenceVac®. There was no significant difference in the GMTs between groups at study day 28 or 56. In addition, there were no significant differences in the safety profile between the three groups. Intercell believes that the data from this trial support the use of the 3 µg dose in children below three years of age. Two additional trials are planned for paediatric populations.

A large pivotal safety, immunogenicity and dose confirmation trial will be conducted in South-East Asia. A total of 1859 children are planned to be enrolled, stratified by age groups ≥ 2 months to < 1 year (IXIARO® group: 125 children, 3 µg dose), ≥ 1 year to < 3 years (IXIARO® group: 638 children, 3 µg dose), ≥ 3 years to < 12 years (IXIARO® group: 400 children, 3 µg or 6 µg dose) and ≥ 12 years to < 18 years (IXIARO® group: 238 children, 6 µg dose). This trial will primarily evaluate the safety profile compared to the licenced vaccines Prevnar® (< 1 year) and HAVRIX® (≥ 1 year). In a subgroup of the study population, immunogenicity of IXIARO® will be assessed for all age groups, and the appropriate dose in children aged 3 – < 12 years will be confirmed. Seropositivity for JE and dengue will be assessed for all children at baseline, but will not be a reason for exclusion from the trial.

A second planned Phase III study will evaluate the immunogenicity and safety of IXIARO® in non-endemic paediatric/adolescent populations. One hundred children with planned travel to JE-endemic regions are planned to be enrolled. The two studies were planned to start in the fourth quarter of 2009 with the first interim safety- and dose-confirmation data expected in Q3 2010. Full results are expected in 2012. (Post-meeting note: in agreement with regulators the paediatric development plans have been recently modified).

In addition, a safety surveillance programme with the American military, is planned in approximately 20,000 subjects. Intercell also plans to investigate the safety and immunogenicity of IC51 in approximately 200 elderly subjects (≥ 65 years of age) and after co-administration with other travellers’ vaccines.
3. Inactivated JE vaccine candidate (JE-PIV, IC52), Biological E (India)

JE-PIV is produced by inactivation of the Vero cell-grown JE virus strain SA 14-14-2. The vaccine is manufactured in a current good manufacturing practice (cGMP) facility of Biological E as a technology transfer from Intercell Biomedical. Biological E is planning a multicentric, parallel randomized (1:1) open label Phase III clinical study in India in healthy ≥ 1 year to < 3 year old children. The primary objective of the study is to demonstrate the non-inferiority of two doses (3 µg) of IC52 compared to three doses of JenceVac® based on the difference in proportion of subjects with a PRNT$_{50}$ ≥ 1:10 achieving a 4-fold rise in anti-JEV neutralizing antibody titres at day 56 post-immunization. Secondary objectives are to compare the immunogenicity of both vaccines at day 28 and 56 (based on GMT), to compare the proportion of subjects seroconverting by day 28, to compare the proportion of subjects achieving a fold rise in anti-JEV neutralizing antibody titres above the seroconversion cut-off value at day 28, and to assess the safety profile of IC52 in comparison with JenceVac®. A separate study will also evaluate the lot-to-lot consistency using three production lots. Successful completion of the study will be the basis for licensure in India, and will provide a JE vaccine produced in Vero cells to meet the requirement for immunization of children and adults in India and other regions of Asia. Biological E plans to apply for WHO prequalification for IC52 immediately upon licensure in India. Submission for licensure in India is expected in the second quarter of 2010, with submission for WHO prequalification expected in the third and fourth quarter of 2010.
JE-CV (trade name IMOJEV®) was originally developed as ChimeriVax®-JE by Acambis, which is now part of Sanofi Pasteur. The JE-CV virus is a live attenuated chimeric virus obtained by replacing the premembrane (PrM) and envelope (E) structural proteins of the yellow fever 17D attenuated virus with those of the attenuated JE virus strain SA 14-14-2. The vaccine is presented as either a 1-dose or a 4-dose vial. The 1-dose presentation is provided as a freeze-dried powder containing ≥ 4.0 log_{10} plaque-forming unit (PFU) in a 3-mL glass vial to be reconstituted with 0.5 mL diluent (0.4% NaCl) for subcutaneous injection. The multiple-dose presentation is under development and will use 0.9% NaCl as diluent. The vaccine contains no preservative or adjuvant.

In preclinical testing, there was no increase in neurovirulence of the vaccine after six serial passages in 3-4 week-old mice. Neurovirulence was less than that observed in monkeys infected with the YF17D vaccine virus. Toxicity and biodistribution testing was done in cynomolgous monkeys. Viremia was detected in 50% of animals at day four, with a peak titre of ≤ 1.9 log_{10} PFU/mL. The virus distribution and viral excretion were determined by quantitative PCR. Virus was detected only at the injection site. The PrM and E sequence of virus recovered from all animals was identical to input virus.

Evaluation of the vaccine was done to determine the risk of dissemination of the vaccine to non-vaccinated populations by mosquitoes. Experimental studies in six different species of mosquitoes determined that JE-CV is unable to replicate in mosquitoes after oral infection, despite the use of a very high inoculum. The effect of JE-CV recombination with wild-type JE virus, West Nile virus, Kunjin virus, dengue viruses, and the YF Asibi wild-type virus was evaluated by using recombinant deoxyribonucleic acid (DNA) technology to create a panel of recombinant viruses. All constructs were attenuated compared with the parent wild-type flavivirus.

More than 2450 adults have received JE-CV in nine clinical trials. The trials were conducted in Australia and the USA and included two Phase I/II trials, five Phase II trials, and two Phase III trials. Protection was assessed based on the commonly accepted protective neutralizing titre of ≥ 1:10 as measured by PRNT_{50} [4]. The PRNT assay was performed using homologous JE-CV virus and a panel of wild-type strains of JE virus.
A single dose of vaccine $\geq 4.0 \log_{10}$ was chosen based on safety and immunogenicity data obtained from two studies. After first vaccination 98.8% seroconverted to JE-CV® at day 28 post-vaccination, and 96.8% of subjects remained seroprotected six months after first vaccination. The protective response was long-lasting as 90% of subjects who received a single dose of JE-CV remained seroprotected at 48 months post-vaccination. Seroprotection was also assessed using heterologous JE viruses. Safety and immunogenicity of JE-CV was further evaluated in a Phase III non-inferiority trial comparing JE-CV to JE-VAX®. A single dose of JE-CV induced seroconversion rates in subjects that were comparable to those induced by three doses of JE-VAX®. Ninety-nine point one percent of subjects vaccinated with JE-CV seroconverted to JE-CV compared with 95.1% of subjects vaccinated with JE-VAX®. There was no significant difference in the safety profile of JE-CV compared with placebo. The most frequently reported reactions in adults were headache, fatigue, malaise and myalgia; frequencies were similar in placebo recipients. Local reactogenicity was less with JE-CV® than with JE-VAX®.

Clinical development in infants: Phase II and Phase III studies have been completed, respectively, in Thailand and the Philippines, evaluating the safety and immunogenicity of JE-CV with the hepatitis A vaccine as a control. The Phase II study enrolled 100 children aged 2–5 years who had been previously immunized with JE-VAX® as part of the national immunization programme, and 200 naive children 12–24 months. Subjects were vaccinated with JE-CV or hepatitis A vaccine in a cross-over design. Neutralizing antibodies were measured 28 days post-immunization using PRNT$_{50}$. All infants who were previously immunized against JE reached a protective level of neutralizing antibodies after JE-CV administration, with 92.8% showing at least a four-fold increase in titres at day 28. Ninety-six percent of naive toddlers reached a seroprotective level of antibody after a single dose of JE-CV. The vaccine was also able to boost seroconversion rates against JE viruses from each of the four genotypes.

None of the children aged 2–5 years had detectable viremia by plaque assay following vaccination with JE-C. Five of 98 children 12–24 months of age had detectable viremia at day four post-vaccination. The mean titre in viremic children at study day four was 20 PFU/mL. The safety profile of the JE-CV was similar to that of hepatitis A vaccine and there were no Serious Adverse Events (SAEs) related to vaccine reported. The Phase III trial also evaluated the safety and immunogenicity of JE-CV and served as a bridging study to compare three lots of JE-CV. Five groups of toddlers 12–18 months were enrolled, with a total of 1100 toddlers receiving JE-CV and 100 receiving the hepatitis A vaccine as control. Consistency was demonstrated for three consecutive lots manufactured in Thailand, as well as bridging with a lot made in the USA. The average seroconversion rate in naive toddlers was 95%.

Sanofi Pasteur filed for licensure in Australia and Thailand in mid 2009. The planned target population for this vaccine is endemic populations. Other studies are planned to evaluate co-administration of JE-CV with measles-containing vaccine and other paediatric combination vaccine. The company has no plans to licence the vaccine in the Canada, Europe, or the USA. The target shelf-life of the vaccine is 36 months when stored at 2°C to 8°C.
5. Live attenuated SA 14-14-2
(Chengdu Institute of Biological Products, China)

Joachim Hombach, on behalf of Mansour Yaïch (PATH), reviewed the co-administration of SA 14-14-2 with measles vaccine which was evaluated in 600 infants nine months of age. This study has been published [5]. Infants were randomly assigned to one of three groups; live attenuated measles vaccine (MV) alone, live attenuated Japanese encephalitis vaccine SA 14-14-2 (LJEV) alone, or MV administered with live Japanese encephalitis vaccine (LJEV). Measles seroprotection rates were high in all three groups, as were JE seroprotection rates, and seroprotection rates were similar across groups for both viruses. A minor drop in measles seroconversion and GMT was observed in the co-administration group. Safety profiles were also similar across all groups. Three-year follow-up is pending for this study. The study will be repeated in Sri Lanka as a requirement for licensure. Two hundred and seventy infants nine months of age will be recruited and randomized to one of three groups: MV alone, LJEV alone, MV and LJEV given concomitantly. A case-control study evaluating the effectiveness of one dose of SA 14-14-2 vaccine against JE was performed in two states in India [6]. Cases were defined by the detection of JE IgM in the cerebrospinal fluid of patients admitted to hospital with an illness consistent with encephalitis. The protective efficacy of a single dose of SA 14-14-2 against JE was 94.5% for the six-month period following vaccination.

Live attenuated SA 14-14-2 vaccine has been registered in the following countries: China (1988), Nepal (1998), the Republic of Korea (2001), Sri Lanka (2003), India (2006) and Thailand (2007). Registration is planned for Bangladesh, Cambodia, the Philippines and Viet Nam in 2009/10. WHO prequalification submission is planned for 2011.
6. Vero-derived inactivated JE vaccine VJE (Biken, Kaketsuken)

In summary, Dr Ichiro Kurane outlined that there are five vaccine companies in Japan, of which two are developing a Vero-cell derived inactivated JE vaccine. Two other manufacturers do not produce Vero-cell derived vaccine at all and the fifth manufacturer has not committed to manufacture.

The Vero cell JE vaccine (VJE) by BIKEN was licensed by the Japanese authorities in February 2009. VJE utilizes the same strain of JE virus (Beijing-1) as the mouse brain-derived vaccine (MBDV). VJE is lyophilized and does not contain thiomersol. Four clinical trials evaluating the safety and immunogenicity of VJE have been completed. BIC001 evaluated two 0.5 mL doses 14 days apart in 17 healthy adults. BIC002 evaluated two 0.5 mL doses one to four weeks apart in 116 healthy children 6–89 months of age compared with 109 children who had received three doses of the MBDV as a comparator. BIC003 evaluated the VJE vaccine as a single 0.5 mL dose in 106 healthy children ages 12–89 months of age; 89 children received three doses of the MBDV as a comparator. The fourth trial evaluated a high dose (2.5 µg in 0.5 mL), a medium dose (1.25 µg in 0.5 mL) and a low dose 0.625 µg in 0.5 mL) in 123, 122 and 119 healthy children, respectively. Two doses of each formulation of the VJE were given 1–4 weeks apart in children 6–12 months of age. Seroconversion rates were 100%, 99.2%, and 95% for the high- medium- and low-dose formulations, respectively. A trial is planned to evaluate a fourth immunization with VJE in children age 10 who have previously received three doses of the MBDV.
7. Dengue vaccine presentation,
Sanofi Pasteur,
14 May 2009

CYD (previously ChimeriVax-DEN) is a live attenuated tetravalent dengue vaccine with genes encoding for the envelope protein of dengue (PrM and E) and the nonstructural and capsid protein of the 17D yellow fever vaccine strain. It is supplied as a powder with solvent for suspension, for administration of a 0.5 mL injection, given either subcutaneously or intramuscularly. The presentation will be as a single-dose vial or a 5-dose vial. The suspension for single dose will be 0.4% NaCl and 0.9% NaCl for the 5-dose vial. The vaccine will be stored at 2°C–8°C. The proposed schedule of vaccination is 0, 6, and 12 months. Each dose contains 5 ± 1 log_{10} CCID_{50} of each serotype. The indication for the vaccine is the prevention of symptomatic dengue disease (covering the spectrum of illness from dengue fever to severe dengue cases) due to serotypes 1, 2, 3, or 4. The target population for the vaccine is toddlers, children and adults living in endemic areas, people working in endemic areas and travellers. The vaccine will be manufactured in France. Vaccine manufacture consists of the production of four vaccine strains coming from four individual seed lots. The same manufacturing process is used for the four serotypes and follows the WHO guidelines [7]. New Vero cell banks were developed without calf serum enabling the vaccine to be produced in serum-free media. Additionally, the vaccine is made without preservatives and is administered without adjuvants.

Preclinical testing: The vaccine demonstrated high genomic and phenotypic stability throughout the manufacturing process. Nine-point mutations were observed across all four serotypes; except for one mutation in the E gene, all of them were located in the non-structural regions of the genome and are likely to reflect viral adaptation to Vero cells. Neurovirulence testing of the vaccine was done by intracerebral injection of mice in accordance with WHO guidelines [7]. CYD vaccine demonstrated no neurovirulence in 3–4 week old mice and was less neurovirulent than the YF-17D virus in 4-day old mice. No increase in neurovirulence was demonstrated after several passages in vitro in suckling mice. Neurotropism was assessed in monkeys in compliance with WHO guidelines [7, 8]. The vaccine was less neurovirulent in monkeys than the YF 17D virus. Hepatotropism was used as a marker for viscerotropism. No infection of the liver by the chimeric viruses was noted in hamsters or monkeys, in comparison to the YF-17D virus which did exhibit a few foci in the liver of monkeys. Based on these experiments, no specific risk of viscerotropism has been identified for the chimeric dengue vaccine candidates.
Environmental safety: Quantitative polymerase chain reaction (PCR) targeting the E/NS1 junction was used to detect viremia in vaccinees [8]. Only low-level viremia of short duration has been detected thus far. Vector transmission studies determined that the chimeric viruses were not infectious to mosquitoes via the oral route. Replication of the chimeric viruses after intrathoracic inoculation of mosquitoes was similar to that of the YF-17D virus and was significantly lower than that of the parent dengue viruses. There was no dissemination of the chimeric dengue viruses to the salivary glands of the mosquitoes, and there was no transmission of the viruses. The risk of increased transmissibility and/or reversion to more virulent viruses, should the unlikely event of recombination between vaccine virus and a wild-type virus occur, was assessed by genetically engineering recombinant viruses [9, 10]. No risk of enhanced transmissibility or reduced attenuation was identified.

Clinical development: Seropositivity was defined as an antibody titre of $\geq 1:10$, as detected by either plaque reduction neutralization $50\%$ (PRNT$_{50}$) or by microneutralization. Virologic assays used in the clinical studies to monitor post-vaccinal viremia and to differentiate dengue vaccination from wild-type infection included dengue NS1 antigen enzyme-linked immunosorbent assay (ELISA), pan-dengue RT-PCR, YF RT-PCR, dengue serotype-specific qRT-PCR, and plaque assay. Thus far, six clinical studies have been completed. Five of those trials evaluated the tetravalent chimeric dengue vaccine.

Safety profile: Data from 318 subjects who received $\geq 1$ dose of vaccine ($5 \log_{10} \pm 1$) in three different Phase I trials determined that the vaccine’s safety profile was acceptable. Flavivirus non-immune and immune subjects were enrolled in Mexico, the Philippines and the United States. There were no SAEs related to vaccination and no mild dengue-like syndrome was observed. The reactogenicity profile was comparable to the control vaccines, with the majority of adverse events being of mild to moderate severity and lasting less than three days. There was no increase in reactogenicity when the vaccine was administered to flavivirus-immune individuals, or when the vaccine was administered to younger subjects (2–11 years was the youngest group studied). No increase in reactogenicity was observed after a second or third dose. Only a low level of viremia was observed, with the dengue serotype four vaccine being the vaccine serotype detected most frequently, followed by vaccine serotype three. Less viremia was detected in children, in dengue-immune subjects compared with non-immune subjects, and in subjects receiving a second or third dose of vaccine. No increase in viremia was detected in yellow fever (YF) virus immune subjects compared to YF naive subjects.

Immunogenicity: In non-endemic populations, a balanced immune response to all four serotypes was achieved after three doses. Children mounted a higher immune response, as did those individuals who had previously received YF vaccine. In endemic populations, a booster effect was noted in subjects previously exposed to wild-type dengue virus, and a stepwise increase of seropositivity rates against each serotype was noted with three doses given as a 0/3/12 month schedule. Two doses given eight months apart induced a similar response in these subjects, as did the 3-dose schedule. In both flavivirus naive and flavivirus primed subjects, the innate and adaptive immune responses correlated with the humoral response and safety profile of the vaccine. Data from these studies provided the rationale for use of a 0/6/12 month schedule in expanded efficacy trials.
Expanded Phase II clinical programme: Five clinical trials for safety and immunogenicity are either ongoing (3) or planned (2) in dengue endemic areas. These studies employ a 0/6/12 month schedule. A randomized, controlled study in 180 healthy children, adolescents, and adults (ages 2–45 years) is being conducted in Viet Nam. A further randomized blind observer-controlled trial is being conducted in Peru in children aged 2–11. A third randomized blind observer-controlled study is being conducted in Singapore where 1200 healthy persons aged 2–45 will be enrolled. No safety signals have been identified in these ongoing studies. Additional Phase II trials in children and adolescents are planned for Central and South America and were scheduled to begin in September 2009.

Efficacy studies: In addition, the first efficacy study has begun in Ratchaburi, Thailand. This study will enroll a total of 4002 children, 2668 of whom will receive the tetravalent dengue vaccine at 0/6/12 months and 1334 of whom will receive placebo as control. The primary endpoint is to assess the efficacy of the dengue vaccine after three injections in preventing symptomatic, virologic-confirmed dengue cases, regardless of the severity, and due to any of the four dengue serotypes. Long-term safety follow-up of subjects will occur up to 48 months after their last vaccination. The evaluation of vaccine efficacy in multicentre Phase III studies is in preparation.

Additional immunogenicity studies: Cell-mediated immunity (CMI) is being evaluated in a subset of volunteers enrolled in CYD28 in Singapore. Th1/Th2 responses were evaluated in 40 adult and 40 adolescent subjects before, and 28 days after the first and third doses of vaccine. In addition, Sanofi Pasteur is interested in assessing the ability of antibodies, induced by the tetravalent dengue vaccine, to neutralize a variety of wild-type dengue virus strains. Recent DEN1-4 field isolates from a variety of endemic areas (Martinique, Puerto Rico, Singapore and Thailand) have been collected and are being amplified in and adapted to mammalian cells for use in a neutralization assay. Studies of monkeys vaccinated with two or three doses of tetravalent dengue vaccine have demonstrated that their post-vaccination sera neutralized these strains.
8. Live attenuated dengue vaccine, GlaxoSmithKline (GSK)

Note: Data from clinical trials TDEN 001–003 of the GSK dengue vaccine candidate are still under analysis by the sponsor. Permission to release of partial clinical data as presented to WHO was not given. The sponsor will disclose the data in peer-reviewed journals once fully analysed.
Alex Kroeger (TDR) presented on the rationale, evidence and status of an effort to revise the reclassification of dengue. With the spread of dengue throughout the world, it has become increasingly evident that patients can have severe dengue without fulfilling the WHO criteria for dengue haemorrhagic fever (DHF). Such cases include:

- dengue with haemorrhagic manifestations but without vascular leakage;
- dengue with shock syndrome, but without fulfilling the four WHO criteria — this can be up to 18% of patients with shock syndrome;
- organ failure is reported in severe disease that does not meet WHO criteria.

For these reasons and in an attempt to simplify the classification and diagnostic algorithm, a reassessment of the WHO dengue case classification has been launched through a large multi-centric prospective clinical study. The DENCO clinical study enrolled patients from study sites with high-level hospitals in Brazil, Cuba, Malaysia, Nicaragua, the Philippines, Thailand, Venezuela and Viet Nam from 2004–2008. Laboratory confirmation of clinically suspected dengue cases was defined by serology (IgM or IgG seroconversion in paired samples) or virus isolation by PCR or culture. Highly-suggestive cases of dengue were defined by serology without virologic confirmation. Analysis for 1724 dengue-positive individuals was presented (1493 from Asia and 231 from Latin America). Of these, 9.2% had dengue shock syndrome (DSS), 35.7% had DHF and 11.8% had dengue fever (DF). Based on current WHO criteria more than 40% of the patients could not be classified without using population haematocrit data. This was reduced to 17% when population haematocrit data were included (9.3% DSS, 41.2% DHF and 55.9% DF).

A revised classification system based on severity scores was presented: category 1 (mild), category 2 (moderate) and category 3 (severe). This system was based on the interventions provided and the care level required. Care level required was defined as: level 1 (in- or outpatient, free to walk around), level 2 (hospitalized, more stringent observation protocol), and level 3 (bedrest, intensive care-unit level observation protocol). Based on these DENCO criteria by interventions, of the 1724 patients enrolled, 13.2% met criteria for severe, 46.8% met criteria for moderate illness and 40% met criteria for mild illness.
Further analysis included the testing of key variables (laboratory and clinical) to distinguish mild/moderate dengue from severe dengue. When classification of severe disease or mild/moderate disease was done using the defined key variables (revised classification), it was found that nine patients who were classified as having severe disease by intervention criteria were classified as mild/moderate disease by the revised classification. In addition, 50 patients who classified as having mild/moderate disease by the intervention criteria were classified as having severe disease by the revised classification. The revised classification can distinguish between severe and non-severe (mild/moderate) dengue with a sensitivity and specificity of around 95%. To better determine who might progress from mild/moderate dengue to severe dengue, an approach was discussed using defined major and minor criteria. This approach is much like the “Jones criteria” to define acute rheumatic fever. The analytical framework for dengue included two or more continuous variables (defined laboratory values, systolic blood pressure) or three or more defined clinical signs or symptoms, or one or more laboratory and two or more clinical variables. The following “warning signs” were identified as predictors of progression: abdominal pain/tenderness; mucosal bleeding; rash; lethargy; low albumin, low platelets, and increased haematocrit. It is planned to validate the study of the revised classification in the clinical practice and surveillance involving 18 countries, further analyse the predictive value of warning signs, and to analyse the signs and symptoms of “probable dengue”.

10. Safety evaluation of dengue vaccines

Anna Durbin (Johns Hopkins University) presented considerations for safety evaluation of dengue vaccines. The *WHO Guidelines for the clinical evaluation of dengue vaccines in endemic areas* (WHO/IVB/12.08) were developed with contributions from regulators, manufacturers, representatives from endemic areas and academic researchers. The guidelines recognize that Phase II and Phase III clinical trials of candidate live attenuated tetravalent dengue vaccines are imminent, and that certain questions remain regarding their safe evaluation. The guidelines established that the only practical efficacy endpoint for these vaccine trials is the reduction of confirmed dengue disease. The guidelines also discussed the need for long-term follow-up of vaccines to determine the risk of developing severe dengue as antibody titres after vaccination wanes. These guidelines focused on later stage trials when follow-up of participants is less intensive.

In early Phase I trials, volunteers are generally followed more intensively and have more clinical and laboratory follow-up. Unfortunately, the methods of follow-up, laboratory studies drawn, and number of visits post-vaccination have varied from trial-to-trial. In addition, the definitions of various adverse events such as neutropenia, thrombocytopenia, fever and seroconversion, have also varied between trials.

To better harmonize the evaluation of these vaccines and the ability to compare vaccines, it was proposed that standard definitions of specific adverse events be developed for dengue vaccines. This could be done in a manner similar to that being currently done for malaria vaccine under the Brighton Collaboration. A group of experts in the field of dengue vaccines would convene to develop a standardized definition of adverse events related to dengue vaccines. This proposal was discussed by the Committee with the representatives from industry. It was felt that the Committee would benefit from a progress report on the Brighton Collaboration’s efforts in establishing standard adverse event definitions for malaria vaccines prior to committing to such an effort for dengue vaccines. It was noted that the Brighton Collaboration has begun developing such recommendations for malaria vaccines.
11. List of participants

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12. References


The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB’s mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director’s Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.