Meeting of the WHO Task Force on Clinical Trials of Dengue Vaccines

Atlanta, GA, USA
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The Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible.

This document was produced by the Initiative for Vaccine Research of the Department of Immunization, Vaccines and Biologicals

Ordering code: WHO/IVB/07.11
Printed: November 2007

This publication is available on the Internet at: www.who.int/vaccines-documents/

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Printed by the WHO Document Production Services, Geneva, Switzerland
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Abbreviations and acronyms

AE  Adverse Events
ALT  Acute Liver Toxicity
AST  Acute Systemic Toxicity
CDC  Centers for Disease Control and Prevention
CMI  Cell Mediated Immunity
CPK  Creatine Phosphokinase
CYD  Chimerax-DEN
DEN  Dengue
DNA  Deoxyribonucleic acid
ECBS  Expert Committee on Biologicals Standardization
ELISA  Enzyme Linked ImmunoSorbent Assay
FDA  Food and Drug Administration
GMT  Geometric Mean Titre
HLA  Human Leukocyte Antigen
IFN  Interferon
IgG  Immunoglobulin M
IgM  Immunoglobulin G
IRB  Institutional Review Board
JE  Japanese Encephalitis
NHP  Non-human Primates
NIBSC  National Institute for Biological Standards and Control
NS  Non Structural
Nt  Nucleotide
PBMC  Peripheral Blood Mononuclear Cell
PBS  Phosphate Buffered Saline
PDK  Primary Dog Kidney
PDVI  Pediatric Dengue Vaccine Initiative
PFU  Plaque Forming Unit
PI  Principal Investigator
prM  Pre-Membrane
PRNT  Plaque Reduction Neutralization Test
RT-PCR  Reverse Transcriptase Polymerase Chain Reaction
<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>SAE</td>
<td>Serious Adverse Events</td>
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<td>SC</td>
<td>Steering Committee</td>
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<td>SCID-HuH</td>
<td>Severe Combined Immunodeficient mouse-human</td>
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<td>TCID</td>
<td>Tissue Culture Infective Dose</td>
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<td>Th</td>
<td>T Helper</td>
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<td>TNF?</td>
<td>Tumor Necrosis Factor Alpha</td>
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<td>TSE</td>
<td>Transmissible Spongiform Encephalopathy</td>
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<td>US NIH</td>
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<td>UTR</td>
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<td>WRAIR</td>
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<td>YF</td>
<td>Yellow Fever</td>
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Special thanks is given to the Chair of the meeting, Dr David Vaughn, as well as to the rapporteur Dr Lewis Markoff. This conference was supported in part by PDVI.
Preface

Introduction

The meeting was opened by Dr Joachim Hombach (WHO), who welcomed participants and summarized the general meeting objectives as follows: (1) to review the current status of vaccine development, (2) to identify common obstacles and to discuss common activities to advance in the field, (3) and to make recommendations to WHO and vaccine developers. More specifically, this meeting had the following main agenda items:

1) Reports from vaccine developers;
2) Discussion of PRNT Guidelines;
3) Discussion of PRNT Validation Panels;
4) General Discussion;
5) Recommendations (closed session).

The outgoing long-serving chair of the task force, Dr Frank Ennis (University of Massachusetts), was thanked for his dedication and leadership, and the new chair, Dr David Vaughn (US Military Infectious Diseases Program) was warmly welcomed.
1. Reports from vaccine developers

Dr Bruce Innis (GSK) discussed the current status and future plans for development of the tetravalent live vaccine (TDEN) based on PDK-cell passaged strains developed at WRAIR. The vaccine is lyophilized and has been shown to be stable for up to 24 months at 2° to 8°C. GSK plans to seek an indication for use in persons 12 months to 45 years of age. The dose is 0.5 ml at 6 months interval via the subcutaneous (SC) route. Each monovalent component of TDEN was recently re-derived by transfection due to the remote risk that the original product(s) might have been contaminated with TSE agents. Clinical data for the monovalent components was obtained using the original product, prior to the re-derivation. Each monovalent vaccine was tested in ~30 adults. TDEN has now been tested in clinical trials over the last 4 years. It has been shown to be safe in adults (N>100), children (N=7), and infants 12 to 15 months of age (N=30). TDEN was highly immunogenic vs DENV2 and DENV4, less immunogenic vs DENV1 and DENV3.

A new IND for TDEN prepared from viruses derived by transfection was filed in 2006, and clinical trials using this preparation were initiated in April 2006. The genomes of vaccine viruses re-derived by transfection were shown to be the same as or similar in consensus nucleotide (nt) sequence to those of the parent viruses. The quasi-species nature of the genomes pre-versus post-transfection were also shown to be similar, as was their level of attenuation in non-human primates (NHP). TDEN001 is a Phase 2 clinical trial designed to compare TDEN derived by transfection to the pre-transfection product and to a placebo, for safety. Adults 18 to 45 years of age are being enrolled. The pre-transfection product will be “formulation 17”, a mixture of the monovalent vaccines that was previously shown to give optimum tetravalent immune responses, compared to a total of 16 other formulations. Endpoints will be solicited symptoms, unsolicited AEs, lab data, and humoral and cellular immune responses. A second Phase 2 trial is slated for 2007 in Thailand. Two formulations of TDEN will be compared to placebo in a 3-arm study in healthy adults ages 20-25 years, N = 120. Volunteers will be screened for prior flavivirus exposure, but not excluded form the study. Endpoints will be similar to the above. Future plans include the production of commercial lots and a study (TDEN003) to be conducted in children down to 12 months of age in a dengue endemic area, with safety and immunogenicity as endpoints.

In the discussion that followed, Dr Innis was asked to explain why the monovalent vaccines were re-derived by transfection (see above), and it was suggested that this also could have been done by plaque-to-plaque purification of each monovalent virus. The rationale for the various formulations of TDEN was also discussed very briefly.
Dr Niranjan Kanese-Thasan of Acambis summarized progress on development of a tetravalent Chimerivax-DEN (CYD) vaccine. Study H050/CYD02 is a randomized, placebo-controlled Phase 1 study of the safety, tolerability, and immunogenicity of the tetravalent candidate in adults 18 to 40 years old. Three groups (N=33 per group) were immunized on day 0 with CYD, YF vaccine, or placebo. After 6 months, the study was unblinded and all subjects received a dose of CYD (N=29 in the CYD group, N=26 in the YF vaccine group, and N=24 in the placebo group). CYD contained 3-4 log10 TCID50/0.5mL of each of the 4 chimeric vaccine candidates. Thus, an estimation of the effect of YF vaccine on the subsequent immune response to and safety of CYD was possible. Safety was determined by use of a diary card for the first 30 days after each dose of vaccine and by a determination of body temperature for the first 21 days. AEs were solicited in interviews conducted every other day up to day 21, and subjects were examined on days 15 and 31 post-inoculation. Subjects were screened for previous flavivirus exposure by PRNT and ELISA. DEN-specific neutralizing antibodies were measured during screening and on days 11 and 30 after each immunization, prior to the second dose of vaccine (at the 6-month time point), and again at 12 months. Viraemia was sought every other day for 21 days post-vaccinations.

There were no deaths and no SAEs related to vaccine. Seven subjects had fever >38°C, 4 after CYD, 2 after YF vaccine, and one in the placebo group. Four fever episodes occurred more than 25 days post-vaccination, suggesting that they were not related to vaccine. The highest temperature recorded was 38.9°C. AEs such as headache, lethargy, fatigue, myalgia, arthralgia, diarrhea, injection site pain, pruritis, swelling, etc., post dose 1, tended to be more frequent in the YF vaccine group than in either of the other two groups. After dose 2 of CYD (for the CYD group) and after dose 1 of CYD in the group that received YF vaccine as a first dose, AEs were in general slightly lower in incidence than for subjects in the placebo group, who were receiving their first dose of CYD. Thus prior exposure to YF vaccine did not have an adverse effect on safety of CYD. After dose 1, viremia due to CYD was more frequent, longer in duration (1.3 days vs 0.3 days on average), and of higher titer than viremia due to YF vaccine administration. For the groups that received a second dose of CYD, viremia was markedly reduced in all parameters. For the YF vaccine and placebo groups who then received CYD, the incidence, duration, and peak titers of virus were comparable to that seen for naïve subjects in the CYD group. Thus prior exposure to YF vaccine did not enhance viremia seen after subsequent immunization with CYD.

For the 33 subjects in the CYD group, 21 (67%), 20 (64.5%), 29 (93.5%), or 25 (80.6%), respectively, had a neutralizing antibody response to DENV-1, DENV-2, DENV-3, or DENV-4, after one dose, where the criterion for a response was a titer of >1:10 against any one of 3 sets of antigens: (i) DENV strains obtained from Mahidol University, (ii) the respective Chimerivax-DEN viruses, (iii) DENV strains obtained from WHO. When 31/33 of these same sera were tested against only the Mahidol University wildtype viruses, a response to DENV-1, DENV-2, DENV-3, or DENV-4 was seen in respectively 58%, 22.6%, 90.3%, or 67.7% of samples. 42% of these sera displayed a trivalent response, and 16.1% displayed a tetravalent one. There was some augmentation of the antibody titers and some recruitment of additional seroconverters after a second dose of CYD, but these phenomena were difficult to assess, because data for the antibody titers 6 months post dose one of CYD, prior to the second dose, were not available.
Among 29 subjects who received two doses of CYD, 3 (11.1%) had a trivalent response and none had a tetravalent response in sera obtained 30 days post-dose 2, using the Mahidol viruses as antigen. There was evidence also that prior exposure to YF vaccine improved the breadth and intensity of the immune responses to CYD.

The discussion centered around the serologic responses late after dose 1 and early after dose 2 of CYD. Unfortunately, the data were not available. In addition, there was a discussion of what approaches are planned to improve the response to the booster dose of CYD and to broaden the response. There was general agreement, however, that it is not unusual to see a relatively limited immune response to a booster dose in clinical trials of live dengue vaccines.

Dr. Jean Lang (SP) spoke about phase 2 clinical trials of the CYD tetravalent vaccine. Objectives are (i) to assess safety in subjects ranging from 2 years of age to adulthood with differing dengue immune status (using a protocol similar to the one described by Dr. Kanesa-thasan), (ii) to assess viraemia adults by RT-PCR, and (iii) to assess the immune response after one, two, or three doses of vaccine. Neutralizing antibodies will be measured by a PRNT50 using the Chimerivax-DEN viruses as antigen in one set of tests and the Mahidol wildtype dengue viruses in another set of tests. In addition, dengue-specific IgM/IgG ratios are being calculated by ELISA and cytokine levels were determined in a cohort of subjects. Plans also include an assessment of cell-mediated immunity (CMI) induced by the vaccine. The first of these Phase 2 trials (CYD04) was conducted in the US in an 18 to 45 year-old adult population that did or will receive three doses of vaccine on a 0, 3-4, 12 month schedule. There were 2 groups of 33 flavivirus-naïve subjects each; group 1 received CYD at both the 0 and 3 to 4-month time points, and group 2 received placebo at 0 and CYD only at 3 to 4 months. In contrast to the Phase 1 trial described above by Dr. Kanesa-thasan, the dose of the monovalent components of the vaccine was increased to ~5 log10 TCID50 each in Phase 2 trials, in hopes of improving the tetravalent immune response.

The second study is being conducted in Latin America. In this study, Group 1 received 3 doses of CYD (@ 0, 3-4, and 12 months); Group 2 received the SP YF vaccine, Stamaril, for dose 1 and this will be followed by two doses of CYD, at the 3-4 and 12 month time points. This was a step-down study, first in 18 to 45 year-olds (N=18), then in 12 to 17 year-old adolescents (N=36), and finally in Mexican children less than 12 years old (N=72). Safety of the formulation was the major criterion for stepping down to lower age groups. The adult part of the study was conducted as described for the phase 2 trial in US adults (see above). In the pediatric phases, blood was drawn for viremia at time points pre-selected based on the data from adults or in case of an AE suggesting that viremia needs to be quantified.

Results of CYD04 for the first 30 days post-dose 1 (the trial in US adults) were described. With regard to local and solicited AEs (including headache, malaise, myalgia, asthenia, pyrexia, injection site pain and swelling, and nausea), Group 1 was different from Group 2 (which received placebo) only in an increased incidence of myalgia and asthenia. Six subjects (18%) in Group 1 had fever, as compared to 4 subjects (12%) in Group 2 who also reported fever. Subjects in Group 1 also reported more injection site pain (18% vs 6%). Small numbers of subjects in both groups had mild transient elevations of AST and ALT after dose 1.
During the same period, two subjects in Group 1 had mild bilirubin elevations, as compared to none in Group 2, and 9 in Group 1 had “leukopenia” as compared to one in Group 2. Data regarding the extent and duration of fever, AST/ALT elevations, bilirubin elevations, and WBCs in subjects with leukopenia were not presented. However, in general it was demonstrated that the increase in dose of CYD from 4.0 log10 TCID50 per serotype to 5.0 log10 TCID50 per serotype was not associated with a significant increase in reactogenicity.

Viremia in subjects in Group 1 of CYD04 was noted on day 0 and again on days 6 through 20 in from about 5% to more than 40% of vaccinees. Peak incidence of viremia was seen on day 8, and the peak titer was 1.46 log10 TCID50/mL. Serotype-specific RT-PCR results demonstrated that most viremia was due to the ChimerivaxDEN4 component of the tetravalent mixture. The incidence of viremia in all subjects in Group 1 was markedly reduced in incidence after a second dose of vaccine. 83% of subjects had a trivalent immune response after two doses of vaccine, while 60% had a tetravalent response. After two doses, GMTs in responders to each of the four serotypes were 35, 81, 29, and 133, respectively, vs DENV-1, DENV-2, DENV-3, and DENV-4, using parental strains.

Dr Lang next clarified the criteria that were used in the phase 2 pediatric trial for stepping down from the adult to the adolescent and eventually to the pediatric cohorts, after dose 1. This involved an interaction between the IRB and the independent data monitoring committee after day 14 safety data had been collected. Then the decision whether or not to proceed was made by the sponsor and the PI based on the independent data monitoring committee recommendation. He also described the study plan in relation to the timing of clinic visits, blood draws, phone calls, etc. The study has already proceeded to the pediatric age group and preliminary safety data post dose 1 were presented. In general, more AEs were seen in the adolescent group than in the adult or pediatric groups, and the overall incidence of AEs were comparable to those seen in the YF vaccinees. AEs were described as “mild”. Apparently no SAEs occurred. CPK (10/84 vs 5/84) and Bilirubin elevations (5/84 vs 0/42) were seen more often in pediatric CYD vaccinees vs YF vaccinees, but AST, ALT, and Creatinine elevations were not different in incidence between the two groups, nor was the incidence of leukopenia. The following conclusions were drawn: (i) the safety profile of CYD at the dose used was satisfactory in adults and pediatric age groups; (ii) there was no increase in reactogenicity after a second dose; (iii) viremia was low in titer, when it occurred; and (iii) immunogenicity after two doses of 5 log10 TCID50 was encouraging.

It was asked whether decreasing the time between dose 1 and dose 2 in the Phase 2 study (3 months) vs the Phase 1 study described by Dr Kanesa-thasan (6 months) might have had some effect on the improvement in immunogenicity that was observed. Dr Lang agreed that this might have been a factor, in addition to the increase in dose of vaccine.
Dr Bruno Guy (SP) addressed plans for assessing the CMI response to the CYD tetravalent vaccine. He briefly summarized how dengue virus infection elicits T cell responses via direct infection of and indirect effects on antigen presenting cells, such as B cells, monocytes, and dendritic cells, and how these early events can impact either severe dengue or a protective response. These considerations were used to determine the most relevant tests to be performed on specimens obtained in the phase 2 trials. Reagents consisted of purified Chimerivax DEN1-4 vaccines, of purified E proteins for DENV-1, DENV-2, DENV-3, and DENV-4, which were produced in *E. coli*, and of peptides that spanned the sequence of NS3 proteins of YF and DEN viruses. Since there is a high conservation of the amino acid sequence of NS3 among DEN viruses, only one set of NS3 peptides was synthesized. Dr. Guy then presented evidence that peripheral blood mononuclear cells (PBMC’s) from YF vaccinees and/or from CYD vaccinees secreted TNFα and IFNγ after stimulation with a specific pool of peptides derived from the YF NS3 protein sequence.

Subjects who had received a live, attenuated DEN vaccine had a similar response to specific pool(s) of DEN NS3 peptides. He then asked which responses were seen after tetravalent CYD vaccination with respect to DEN serotype-specific T and B cells, DEN serotype-crossreactive T and B cells, and YF 17D NS protein specific T and B cells, using cell samples obtained before and 28 days after vaccination, from the US-based Phase 2 trial in 66 adults (CYD04; see above). Serum samples were also collected every two days in the first two weeks following vaccination. Results showed that there were no changes in serum pro- and anti-inflammatory cytokines (interleukins 1β, 6, 8, 10, and 12p70, IFNα and γ, and TNFα over the observation period. However, an IFN γ/TNFα response to two sets of pooled peptides (pools C and F) derived from the YF 17D NS3 protein sequence was demonstrated for CD8+CD3+ cells from one vaccinee’s PBMCs. In sum, CD8+CD3+ T cells from 9 of 22 tested subjects in Group 1 were stimulated after one or two stimulations to secrete IFNγ and TNFα after exposure to at least one set of pooled peptides (usually pool C, but responses were also seen to pools B, D, and F). Two of 8 subjects in Group 2 had a similar response after one immunization but only to pool C of peptides. Candidate T cell epitopes in the linear sequence of the YF 17D NS3 protein that were represented in peptide pools B and C were identified. A previously identified HLA B35 epitope in the YF NS3 was present in peptide pool D, which was in general not stimulatory for T cells from vaccinees in this study.

Some data on DENV serotype-specific stimulation of cytokine levels after one or two doses of CYD was also shown. Increased secretion of IFNγ but not TNFα was seen after dose 1. Levels returned to baseline by month 4 prior to dose 2 and a second increase was seen after dose 2. In general, serotypes 2 and 4 were more stimulatory than serotypes 1 and 3. There was no increased secretion of Th2 cytokines. Dr. Guy concluded that CYD vaccine is able to stimulate Th1 and 17D NS3-specific CD8 T cell responses in vaccinees. While the response is somewhat dominated by DENV-2 and DENV-4 after one dose, there is broadening after 2 doses.
In the subsequent discussion, it was noted that assays had been conducted using the DENV NS3 peptides and that T cells from vaccinees can discriminate between the two sets (YF versus DENV). Assays were conducted doing usual 4h in vitro T cell restimulation. The relevance of the response to the YF 17D NS3 was questioned, but relevance may become evident after sufficient data for a large number of vaccinees have been collected. It could be that YF17D cellular response shows a positive bystander effect on DEN-specific E responses. In that context, it was noted that there are ample data to confirm the finding that YF 17D elicits a strong and durable memory response. Dr Guy also clarified that the specificity of the T cell response to YF versus DENV peptides is maintained post-second dose, even though it broadens somewhat.

Dr Anna Durbin of Johns Hopkins University School of Public Health presented data on the live, attenuated “Δ30” vaccines developed at the US National Institute of Health (NIH). These vaccines are all based on a 30-nt deletion of a conserved stem-loop in the 3’ untranslated region (UTR) of the DENV genome, created by site-directed mutagenesis. The parent virus is based on a DENV-4 infectious DNA, and two sets of vaccine candidates for each of the four serotypes exist; in one set infectious DNAs for DENV-1, DENV-2, and DENV-3 viruses have been separately engineered to introduce the delta-30 mutation. In a second set, the prM and E genes of DENV-1, DENV-2, and DENV-3 have been substituted for the DENV-4 prM and E in the context of the original DEN4Δ30 backbone. The original DEN4Δ30 virus was initially studied in humans at a dose of 10^5 pfu and was found to be well tolerated except for a low incidence of fever, rash, low grade neutropenia, and mild, transient ALT elevations. Eventually, a dose de-escalation study was done in 4 cohorts of 20 subjects each, who received doses of 10^5, 10^3, 10^2, or 10^1 pfu, respectively. Viremia was seen in most of the recipients of the highest dose and 5/20 had mild ALT elevations, but at lower doses viremia was less frequent and of lower peak titer, and abnormal ALT values were no longer seen. These improvements in safety were seen without a parallel sacrifice in immunogenicity. Thus the standard dose of Δ30 vaccines has been set at 10^3 pfu, which can be adjusted up or down as and where necessary for a given serotype in a tetravalent formulation.

Dr Durbin went on to describe the standard approach for studying rDENAΔ30 viruses in Phase 1 clinical trials. These are placebo-controlled, double-blind trials conducted in flavivirus-naïve adults 18 to 50 years old. Twenty subjects of 28 selected are randomly assigned to receive vaccine, and 8 receive placebo. Temperature is recorded for 16 days, and during that time subjects are seen in clinic for physical examination and safety laboratory studies, as well as for blood draws to detect viremia. Neutralizing antibody titers are measured at 0, 28, 42, and 180 days post-vaccination. Local reactogenicity and solicited AEs are also recorded by usual surveillance methods. Details of the Phase 1 trial of rDENAΔ30 vaccine were presented. This vaccine caused mild fever, rash, CPK elevations, and low grade neutropenia in a minority of vaccinees in excess of the incidence in placebos. ALT elevations were not seen. At least some incidences of neutropenia (WBC <1500) were shown to be a function of the baseline WBC prior to vaccination, i.e., false-positives. DENAΔ30 vaccine was highly immunogenic; 19/20 subjects seroconverted with a GMT = 287 at day 180 post-vaccination. Plans are in place to evaluate a 2-dose regimen for this vaccine and to determine the mean human infectious dose.
The construction and Phase 1 trial of the DEN2/4Δ30 chimeric vaccine candidate were presented next. This vaccine elicited only very low levels of local reactogenicity, primarily only local erythema. Eleven of 20 vaccinees were viremic; nine had asymptomatic rashes. Low grade transient neutropenia was seen in 7 subjects, and ALT elevations were seen in 3. Once again, neutropenia was shown to be a function of the pre-vaccination WBC count. DEN2/4Δ30 vaccine was not different from DEN1Δ30 and DEN4Δ30 vaccines in a summary of all AEs. All vaccinees seroconverted, and GMT 180 days post-vaccination was 84 (down from 147 on day 28).

Finally, Dr Durbin reported on attempts to reduce reactogenicity by introducing additional mutations into the DEN4Δ30 genome, particularly with respect to ALT elevations. She discussed charge-to-alanine mutagenesis of the NS5 gene, which led to the selection of DEN4Δ30-200,201 virus and the selection of another mutant in tissue culture, DEN4D30-4995, which bears a point mutation in the NS4B gene. DEN4Δ30-200,201 virus is attenuated in SCID-HuH7 mice and rhesus and is highly immunogenic in NHP. Accordingly, DEN4D30-200,201 vaccine was used in a phase I clinical trial at a dose of 10^5 pfu. No viremia was detected, and no vaccinee had an ALT elevation. Hence, the 200, 201 mutations conferred additional human attenuation on the DEN4D30 backbone. However, only 7 of 20 vaccinees seroconverted, and these subjects had a GMT of neutralizing antibodies on days 28 and 42 that was about 10-fold lower compared to that elicited by an equal dose of DEN4D30 parent vaccine virus. DEN4D30-4995 vaccine was also shown to be attenuated in SCID-HuH7 mice and rhesus and to be immunogenic in rhesus. A Phase 1 trial is planned for April 2007. A list of clinical trials planned for 2007-2008 was presented, laying out the further characterization of monovalent candidates and their progression into two tetravalent formulations.

During the discussion Dr Durbin noted that all clinical data so far has been obtained in flavivirus naïve subjects and that the impact of pre-existing immunity is therefore not known. In relation to the observed AE’s, viraemia was not temporally associated with rash, neutropenia and other AEs. She further noted that the test of the DENV-2 and DENV-3 serotype candidate vaccines will be done using the rDEN4D30 backbone and the prM and E genes of those two serotypes at an initial dose of 10^3 pfu.

Dr Daniel Stinchcomb (Inviragen) presented efforts of the company to bring dengue vaccines based on PDK-53, a DENV-2 vaccine candidate that is derived from an infectious DNA, into clinical evaluation. Chimeric DENV-1, DENV-3, and DENV-4 vaccine candidates have been generated based on this infectious DNA. So far, all 4 vaccines have been shown to be safe (i.e., attenuated) and immunogenic in both mice and monkeys. In monkeys, antibody titers against DENV-1 and DENV-2 are in general higher than those obtained against DENV-3 and DENV-4. Mutations required for the attenuation phenotype of PDK53 with respect to the wildtype DENV-2 parent virus were located in the 5’ noncoding region and NS1 and NS3 gene segments. The vaccines replicate to high titer in tissue culture substrates suitable for vaccine manufacture, and mutations necessary for attenuation have been shown to be stable after multiple passages of virus, when all 3 mutations are present in the initial virus genome. These vaccines are being developed by a partnership among Inviragen, the CDC Division of Vector-borne Infectious Diseases, the University of Wisconsin (for primate studies), and Shantha Biotech, India (for manufacturing). Clinical trials are planned for and in endemic countries.
During the discussion, Dr Stinchcomb clarified that the company also pursues an indication for the travelers market. In relation to genetic stability studies, they have thus far only been done in vitro. In relation to the clinical development, the plan is to proceed as rapidly as possible to testing of tetravalent formulations. It was also speculated that this vaccine approach might suffer from virus interference, once tetravalent formulations are administered. While trials in rhesus monkeys had failed to elicit any evidence for interference among serotypes, it was pointed out that rhesus monkeys have not historically been a good model for assessing interference and immunogenicity, as it is observed in humans.

Dr Kevin Porter of the US Naval Medical Research Center presented a Phase 1 clinical trial of a DENV-1 DNA vaccine. The plasmid DNA vaccine expresses the DENV-1 prM and E genes under control of the CMV immediate early promoter. A GMP preparation of the DNA was contract-manufactured and safety tested. The human dose is 5 mg or 1 mg of DNA in PBS. Toxicity and biodistribution studies were done successfully in New Zealand White Rabbits and in mice, respectively. The Phase 1 trial was an open-label dose escalation study conducted in 24 flavivirus naïve subjects age 18 to 50; 12 received the 1 mg dose initially, and 12 additional subjects then received the 5 mg dose. Vaccine was given at 0, 1, and 5 months via a Biojector device. Local and solicited AEs were graded on a scale of 1 to 4 for severity, as per US FDA guidelines. There were essentially no AEs except for mild fatigue in one or two subjects, and pruritis, induration, and tenderness at the injection site. Several subjects had fairly significant CPK elevations, but most were attributable to vigorous exercise. Other laboratory results were within normal limits. Results of an ELISA for DENV antibodies were shown for 5 subjects who received the 1 mg dose(s) of vaccine, and two had a response. Results of PRNTs and other immunological assays were not presented. The second phase of the study, in which subjects will have received three doses of 5 mg of the vaccine, has not yet been completed.

In relation to the Biojector device, Dr Porter noted that it was used without a spacer, and delayed onset of erythema at the injection site was not observed. There is a limit of volume that can be administered by the device (1ml), which poses problems once tetravalent formulations will be tested. There was also concern that the relatively large volume of 1ml might induce pain. The use of adjuvants to reduce volume and dose of DNA was addressed, but this avenue is currently not pursued by the PI as these products are proprietary. Another discussion item was the absence of detectable neutralizing antibodies after low dose vaccination. One participant suggested that the relatively poor immunogenicity of the vaccine at this dose might be improved by enhancing the efficiency of cleavage at the prM-E site via site-directed mutagenesis of the plasmid.

Dr Beth-Ann Coller of Hawaii Biotech presented information on the development of a tetravalent, adjuvanted E subunit vaccine. This vaccine also includes DENV-2 NS1 protein, in the belief that addition of this antigen enhances the immune response, as suggested by mouse data. The protein antigens are expressed in the Drosophila S2 system. The GMP manufacturing of the product was described, as was the preclinical data that was used to optimize the formulation and evaluate the adjuvant effect. Filling and finishing of the final formulation are planned for Spring 2007 in order to launch the clinical phase. Results of a 36-monkey preclinical trial were used to evaluate a saponin-based adjuvant, based on immunogenicity and
protective efficacy of the vaccine. Three groups of 12 monkeys each were given 1g of 80% E, i.e., E bearing a carboxy-terminal deletion of its transmembrane region, of each serotype plus 60 g of a nondisclosed adjuvant called “D” (Group 1), or the same mixture plus 0.1 g DENV-2 NS1 (Group 2), or adjuvant alone (Group 3), respectively, on days 0, 60, and 120. Three monkeys per group were challenged with each of the four serotypes of DENV 5 months post-vaccination. Monkeys in Groups 1 and 2 had vigorous neutralizing Ab responses to DENV-2 and DENV-3 (testing for DENV-1 and -4 antibodies is ongoing). After challenge, monkeys in Group 1 had no viremia. Only one monkey in Group 2 had viremia (after DENV-1 challenge), and 11/12 monkeys in Group 3 developed viremia after the challenge. All challenges were done at 10^5 pfu. After this study was completed, the company changed the adjuvant to be used in their formulation for commercial reasons to a new saponin-based product, GPI-0100, which has been evaluated in mice so far and is currently being used and tested in the context of a subunit West Nile vaccine. The above described monkey study is currently being repeated using GPI-0100, instead of adjuvant D. Dr Coller further described an in vivo potency assay for the candidate vaccine, using Swiss Webster mice. This test will support lot release. Phase 1/2 clinical trials are planned for the third quarter of 2007. WHO-recommended cells and virus strains will be used for the PRNT.

During the discussion the value of adding NS1 to the formulation using GPI-0100 was questioned. Testing is ongoing and the impact is to be confirmed. It was further explained that the clinical development plan foresees a direct move into a tetravalent formulation and initial trials will be done in Hawaii.
2. Update: Guidelines for the dengue plaque reduction neutralization test

Dr John Roehrig (US CDC, Ft Collins) presented on progress towards the development of WHO Guidelines for the Plaque Reduction Neutralization Test (PRNT) with Dengue Viruses. This effort emerged from several previous consultations held by WHO and the Pediatric Dengue Vaccine Initiative (PDVI), with the aim of harmonizing PRNT methodologies to make results between laboratories more comparable, and to establish PRNT as the gold standard against which other tests can be validated. For the purpose of developing the guidance document, an ad hoc working group had been called upon, with all major dengue vaccine developers being represented. There are now standardized WHO Vero cells and virus strains available for testing, and one polyvalent dengue serum standard (NIBSC code 02/186) has been proposed as an international standard of 100 units for each subtype. More efforts are under way to collect, test and calibrate dengue sera as international reference reagents (see below). Dr Roehrig emphasized that the objective guidelines is to achieve harmonization and to control the key assay variables, but not to prescribe a standard protocol. The effort is supported by PDVI.

A second draft of the PRNT Guidelines were presented at the meeting, and participants were invited to submit their comments in writing to Dr Roehrig. The document is structured into a general scientific introduction, a detailed discussion of the assay conditions, preparation of materials and readout, the presentation of minimal requirements for assay performance, and a brief discussion of novel tests to measure virus neutralization. In terms of process to finalize and approve the document, it is expected that after receipt of the comments on the second draft, the final draft will be peer reviewed once more, before being submitted to the WHO expert committee on biologicals standardization (ECBS) for final approval. The document should be published, both online and in paper version, under WHO label.
3. Update: Establishment of a dengue serum panel for assay validation

Dr Harvey Artsob (Public Health Agency of Canada, Winnipeg) gave an update on the production of dengue reference sera for use as PRNT validation panels. The goal of this project is to provide a serum panel for each of the four serotypes with specimens that exhibit a robust measure of neutralizing activity against which to assess the PRNT. Thus the sera could be used to validate novel neutralization assays under development and for self-assessment by labs. It will primarily be made available to vaccine developers. Together with the PRNT guidance document, these reagents should allow to further reduce the between laboratory variability of assays. At the moment, the panel is not intended for calibration as reference standards, although this could be considered dependent on the quality of the samples. Based on previous consultations with vaccine developers, it was concluded that the panel is to consist of monotype sera against all 4 serotypes, polyvalent sera, sera from vaccinees, as well as sera from naturally infected subjects. The sera should ideally contain a range of antibody titers and have been derived from subjects at least 6 months post-exposure. Non-dengue flavivirus antibody-positive sera (especially for YF, JE, WN) would be desirable. In the following, the sources of sera were discussed. The original plan was for about half the sera to come from vaccinees and half from naturally exposed subjects. Monotypic sera may be donated from non-endemic areas, and travel clinics have been contacted for that purposes. Vaccine developers have offered to contribute monotypic sera from vaccinees. Negative control sera are to be collected from dengue non-endemic countries. Labs at Mahidol University in Thailand and at the CDC in Puerto Rico will characterize the incoming samples. Dr Artsob raised the practical difficulties of moving forward that project that requires commitment by the scientific community and management of the ethical and other aspects.

During the discussion, questions were raised regarding the provenance of the polyvalent sera, and whether pooled monovalent sera could be acceptable. While there were different opinions, a general consensus was reached that it would be preferable, if possible, to obtain polyvalent sera from individuals who had been exposed to multiple dengue infections. To simplify the process of assembling the serum panel, it was suggested to use leftover samples, if available in sufficient amount. This was accepted as a viable interim strategy until suitable donors had been identified and ethics clearance obtained. Nevertheless, this strategy also requires that samples had been collected with proper consent and the mention to use sera for research and diagnostic purposes. The group concluded that 1-10ml of sera would be acceptable to start the exercise. The group also agreed that the samples should be characterized by 2-3 laboratories, including the CDC branch in Puerto Rico, the Mahidol centre for vaccine development, and if possible the Walter Reed Army Institute of Medical Research.
Dr Hombach concluded this part of the meeting by mentioning an ongoing project to revise guidelines for population based clinical trials of dengue vaccines. For that purpose, a drafting group has been put together under the leadership of Dr Robert Edelman (University of Maryland) that was scheduled to meet the coming day (November 12). The stakeholder group contributing to the revision of the documents includes most vaccine developers and academic experts. Several iterations are expected to come to a final document. For the coming meeting, expected discussion items include (i) severe dengue, (ii) clinical trial endpoints, (iii) the subject and nature of bridging studies, (iv) Phase 2b studies, and (v) phase 4 trials. It is expected that a final draft could be discussed at the next Steering Committee meeting in April 2007, with endorsement by ECBS in Fall 2007.
4. General discussion

Dr Alan Barrett (University of Texas, and Chair of the WHO Steering Committee on Flavivirus vaccines) noted the considerable progress made in the previous year and asked how the Steering Committee could help vaccine development in the future. Participants agreed that current emphasis of work in relation to the production of guidelines and reference materials was of high added value to vaccine developers and should be pursued. One additional issue noted was the identification of suitable sites in endemic areas for clinical evaluation of dengue vaccines. PDVI had established itself as a leader in this area through its effort to establish and support a network of field sites in various countries suitable both for longitudinal epidemiological studies and clinical trials. It was noted that public sector support was in particular relevant to academic or smaller companies and that support to these trials had to be negotiated on a case by case basis.

Another discussion item centered on the collection of PBMC and the measurement of CMI in the course of vaccine trials. Two main arguments were put forward: (1) to better understand the role of CMI in vaccine efficacy, and (2) have relevant biological material prospectively available from trials of tetravalent formulations in endemic areas, in order to be able to assess the etiology of unanticipated and possibly rare adverse events. While the first objective is being pursued by vaccine developers, as presented at this meeting, it was felt that the prospective collection of specimen and their storage for future analysis was insufficiently addressed in current discussions, and should be reflected in the respective WHO documents. In order to manage cost and logistics of such an exercise, it was proposed that for the present it would be sufficient to collect and store specimens, pre- and post-vaccination, during Phase 3 trials only.
5. Closed session and final recommendations

The meeting was then closed to representatives of commercial entities for the formulation of recommendations and any other matters. The closed session took place among members of the Steering Committee and Temporary Advisers.

Recognizing the value of the Task Force, participants recommended that more efforts should be made to increase participation from small entities involved in the development of dengue vaccines, in particular from developing countries. The question of advancing promising dengue candidates that currently lack a commercial sponsor into clinical testing was addressed to both PDVI and WHO, but both Organization representatives considered this outside their financial capabilities or core mandate. However, both Organizations could take a lead in brokering suitable development partnerships. Participants also felt that WHO should soon consider consultations on programmatic aspects of dengue immunization, in particular the suitable age of vaccination. Participants encouraged WHO to lead on the key expectation from clinical trials, and to inform both regulators and vaccine developers early on.

In summary and conclusion the following recommendations were made:

- WHO should continue and bring to completion its work on trial guidelines, assay harmonization and provision of standard reagents.
- WHO, together with PDVI should facilitate the early phase clinical evaluation of dengue vaccine candidates in endemic countries.
- WHO should, through the dengue vaccine guidelines that are being developed and other generic documents, propose uniform approaches to establish vaccine safety.
- Vaccine developers should systematically assess cell-mediated immunity in the course of Phase 2 clinical trials, and consider the prospective collection of PBMC from participants of efficacy trials for assessment of infrequent severe adverse events.
- WHO to provide guidance on the above subject and to draft a points-to consider document.
- WHO to encourage emerging vaccine developers, in particular from developing countries, to participate in the WHO task force.
Annex 1:
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The World Health Organization has managed cooperation with its Member States and provided technical support in the field of vaccine-preventable diseases since 1975. In 2003, the office carrying out this function was renamed the WHO Department of Immunization, Vaccines and Biologicals.

The Department’s goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. Work towards this goal can be visualized as occurring along a continuum. The range of activities spans from research, development and evaluation of vaccines to implementation and evaluation of immunization programmes in countries.

WHO facilitates and coordinates research and development on new vaccines and immunization-related technologies for viral, bacterial and parasitic diseases. Existing life-saving vaccines are further improved and new vaccines targeted at public health crises, such as HIV/AIDS and SARS, are discovered and tested (Initiative for Vaccine Research).

The quality and safety of vaccines and other biological medicines is ensured through the development and establishment of global norms and standards (Quality Assurance and Safety of Biologicals).

The evaluation of the impact of vaccine-preventable diseases informs decisions to introduce new vaccines. Optimal strategies and activities for reducing morbidity and mortality through the use of vaccines are implemented (Vaccine Assessment and Monitoring).

Efforts are directed towards reducing financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies (Access to Technologies).

Under the guidance of its Member States, WHO, in conjunction with outside world experts, develops and promotes policies and strategies to maximize the use and delivery of vaccines of public health importance. Countries are supported so that they acquire the technical and managerial skills, competence and infrastructure needed to achieve disease control and/or elimination and eradication objectives (Expanded Programme on Immunization).