Vaccine xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and immunogenicity of three tetravalent dengue vaccine formulations in healthy adults in the USA☆

Gustavo H. Dayan[a,*], Manoj Thakur[a], Mark Boaz[a], Carol Johnson[b]

[a] Sanofi Pasteur Inc., Route 611, Discovery Drive, Swiftwater, PA 18370-0187, USA
[b] Clinical Research Consultants, 3100 Lorna Road, Hoover, AL, USA

ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 19 July 2013
Accepted 27 August 2013
Available online xxx

Keywords:
Clinical trial
Dengue
Formulation
Immunogenicity
Vaccine

ABSTRACT

Background: A candidate recombinant, live-attenuated, CYD tetravalent dengue vaccine (CYD-TDV) has recently demonstrated immunogenicity, efficacy and good tolerability. This study was performed to evaluate three CYD-TDV formulations in adults.
Methods: This was a randomized, double-blind, multicenter, phase II trial. The vaccine formulations were: CYD-TDV 5555 (≈5 $\log_{10}$ tissue culture infectious dose 50% [$TCID_{50}$] of serotypes 1–4); CYD-TDV 5553 (≈5 $\log_{10}$ $TCID_{50}$ of serotypes 1–3 and ≈3 $\log_{10}$ $TCID_{50}$ of serotype 4); and CYD-TDV 4444 (≈4 $\log_{10}$ $TCID_{50}$ of serotypes 1–4). Vaccinations were administered at 0, 6 and 12 months. Immunogenicity was assessed using the plaque reduction neutralization test.
Results: In total, 260 individuals were enrolled. The 5555 formulation elicited a superior serotype 4 response versus the 5553 formulation, with seropositivity rates of 89.7% and 58.3%, respectively, after the second dose (between-group difference 31.4%; 95% confidence interval 18.2–43.2). After each of the three doses, seropositivity rates for serotypes 1–3 were numerically highest with CYD-TDV 5553 and lowest with the 4444 formulation; seropositivity rates for serotype 4 were similar with the 5555 and 4444 formulations, and much lower among recipients of CYD-TDV 5553. Geometric mean titers followed the same pattern as that seen with seropositivity rates. Safety/reactogenicity results were similar for all three vaccine formulations, although the percentage of participants reporting solicited injection site reactions was lower with CYD-TDV 4444 than with the other two formulations. All serious adverse events were unrelated to vaccination.
Conclusions: Reducing the dose of serotype 4 antigen (5553 formulation) creates an imbalance in the immune response to CYD-TDV. Immune responses to CYD-TDV 5555 were slightly higher than to the 4444 formulation. Development of CYD-TDV 5555 has subsequently been pursued.

1. Introduction

Dengue has been described as the most important mosquito-borne viral disease affecting humans [1]. No licensed vaccine or specific treatment exists for dengue, and preventive measures based on integrated vector control have shown limited effectiveness and sustainability [1–3].

Development of a dengue virus (DENV) vaccine has proved challenging. Ideally, protection is needed against all four serotypes, although in vivo interference between the serotypes has presented challenges to achieving a balanced immune response. Clinical studies in the early 2000s with alternative tetravalent dengue vaccine (TDV) formulations based on the live attenuated vaccines developed at Mahidol University, Thailand, showed immunodominance of serotype 3 [4–6]. This vaccine candidate was also associated with the occurrence of dengue-like syndromes after vaccination. Formulation related interference challenges were also observed in another study conducted with a different cell-passaged live attenuated TDV, in this example serotype 1 was immunologically dominant [7].

A recombinant, live-attenuated CYD tetravalent dengue vaccine (CYD-TDV), formulated with ≈5 $\log_{10}$ tissue culture infectious dose 50% ($TCID_{50}$) of serotypes 1–4 (5555 formulation), appeared to solve these issues. CYD-TDV contains four recombinant viruses (CYD-1–4), each with genes encoding pre-membrane and envelope

Abbreviations: CYD-TDV, recombinant live-attenuated tetravalent dengue vaccine; $PRNT_{50}$, plaque reduction neutralization test; RT-PCR, reverse transcriptase-polymerase chain reaction; $TCID_{50}$, $\log_{10}$tissue culture infectious dose 50%.

☆ National Clinical Trials Registration Number: NCT00617344.

* Corresponding author. Tel.: +1 570 957 2952; fax: +1 570 957 0934.
E-mail addresses: Gustavo.Dayan@sanofipasteur.com (G.H. Dayan), Manoj.Thakur@sanofipasteur.com (M. Thakur), Mark.Boaz@sanofipasteur.com (M. Boaz), Jakeschild3@gmail.com (C. Johnson).

0264-410X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2013.08.088

proteins of one of the DENV serotypes, and the attenuated yellow fever 17D vaccine virus [8,9]. Clinical studies have demonstrated that this vaccine is immunogenic for all four serotypes and is well tolerated and, unlike early live attenuated TDV, does not induce dengue-like disease [10–17]. The immune response after multiple doses of CYD-TDV is balanced across the four serotypes, although the antibody response to serotype 4 appears dominant at least after initial vaccinations, and serotype 4 viremia is frequently detected [10,13,15].

The present study was conducted in adults to compare the 5555 CYD-TDV formulation with two alternative formulations. First, a formulation with a lower CYD-4 virus dose (5553 formulation) was assessed because of the immunodominance of serotype 4 and promising simian data [18]. The second comparator (4444 formulation) was chosen to investigate a 1 $\log_{10}$ reduction in dose, thereby increasing the capacity of vaccine production.

2. Methods

2.1. Trial design and participants

This randomized, double-blind, multicenter, phase II trial was conducted in 5 sites the USA (2 in California, 1 in Alabama, 1 in Missouri, and one in New Orleans) between 17 April, 2008 and 14 December, 2009. Healthy adults aged 18–45 years were randomized to receive one of the following three formulations: CYD-TDV 5555 ($\approx$5 $\log_{10}$ tissue culture infectious dose 50% [$TCID_{50}$] of serotypes 1–4); CYD-TDV 5553 ($\approx$5 $\log_{10}$ $TCID_{50}$ of serotypes 1–3 and $\approx$3 $\log_{10}$ $TCID_{50}$ of serotype 4); and CYD-TDV 4444 ($\approx$4 $\log_{10}$ $TCID_{50}$ of serotypes 1–4). CYD-TDV batch numbers were: 5555 formulation, S4168 and S4237; 5553 formulation, S4161; 4444 formulation, S4160. All three formulations were administered by subcutaneous injection, in the deltoid region of the arm.

Female participants of childbearing potential were required to use effective contraception (or remain abstinent) from 4 weeks before the first vaccination until 4 weeks after the last vaccination. The main exclusion criteria were pregnancy or breastfeeding, congenital or acquired immunodeficiency, chronic illness that could interfere with the study, receipt of any vaccine within 4 weeks before the first study vaccination or 4 weeks after each of the study vaccinations, laboratory test abnormalities (complete blood count, platelets, liver function tests, serum creatinine, creatine phosphokinase) at screening, reported history of flavivirus infection as reported by the participant, and previous vaccination against flavivirus diseases (including Japanese encephalitis [JE], tick-borne encephalitis, and yellow fever).

All participants provided signed informed consent. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice and the International Conference on Harmonisation guidelines. Appropriate local ethical committee approval was obtained.

At each site, participants were assigned sequential study identifier numbers and the vaccine was determined by a randomization list generated using SAS® version 8.2 or higher. Randomization was performed using the permuted block method. Twenty-five individuals from each group were randomly selected for viremia assessment.

Six clinic visits were scheduled after screening, three for vaccinations at 0, 6 and 12 months, and three for assessment 30 days after each vaccination. Blood samples were collected at each 30-day post-vaccination visit, and at screening. Four additional visits were scheduled for individuals undergoing viremia assessment, 1 and 2 weeks post-doses 1 and 2.

2.2. Immunogenicity assessment

Neutralizing antibody levels were measured by the plaque reduction neutralization test ($PRNT_{50}$) at screening and 30 days post-doses 1, 2 and 3 [19–21]. The challenge viruses in the $PRNT_{50}$ were the parental dengue viruses of the four recombinant CYD vaccine viruses (i.e., DENV-1 strain PUO-359, DENV-2 strain PUO-218, DENV-3 strain PaH881/88, and DENV-4 strain 1228).

Serial two-fold dilutions of heat-inactivated serum were mixed with a constant challenge dose of each DENV serotype and inoculated into a 24-well plate of confluent Vero cells. After adsorption, cell monolayers were overlaid and incubated. Cells infected with DENV were indicated by formation of plaques. Neutralizing antibody titers were calculated as the highest reciprocal dilution (1/dil) of serum at which $\geq$50% reduction in viral plaque count was observed ($PRNT_{50}$), compared with the mean count obtained with dengue antibody negative control serum. The lower limit of quantitation of the dengue $PRNT_{50}$ was 10 [21]; samples with titers $\geq$10 were considered seropositive. A microneutralization test was initially planned to assess immunogenicity, but its sensitivity to DENV-2 antibodies was too low (data not shown). Therefore, samples were retested using the $PRNT_{50}$ assay, the laboratory standard for assessing dengue virus neutralizing antibodies according to the World Health Organization (WHO) [22]. $PRNT_{50}$ has been optimized and validated in line with WHO recommendations [21].

2.3. Safety and reactogenicity

After each vaccination, adverse events (AEs) were recorded during a 30-min observation period, and using diary cards for 7 days (injection site reactions), 14 days (systemic reactions), and 28 days (unsolicited AEs). Serious AEs (SAEs) were monitored throughout the study.

Solicited injection site reactions were pain, erythema and swelling, whereas solicited systemic reactions were fever, headache, malaise, myalgia and asthenia. An intensity scale (grades 1–3, from mild to severe) was applied to all AEs. All solicited AEs were considered to be vaccine-related. Unsolicited AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) and were classed by system organ classes (SOC) and preferred terms (PT) [23]. The investigator determined whether unsolicited AEs were vaccine-related (AEs considered as related were termed 'adverse reactions'). Biological safety (complete blood count, platelets, liver function tests, serum creatinine, creatine phosphokinase) was assessed at screening and 30 days post-doses 1, 2 and 3. Biological safety in the viremia assessment group was also evaluated at 7 and 14 days post-doses 1 and 2.

2.4. Viremia assessment

Serum samples were tested for CYD dengue vaccine viremia at 7 and 14 days post-doses 1 and 2. Non-serotype-specific viremia was detected by reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted from the serum and RT-PCR was conducted using primers from a yellow fever core gene sequence. For yellow fever RT-PCR positive samples, CYD serotype-specific viremia was detected by four RT-PCRs. RNA was extracted from the serum and RT-PCR was undertaken with serotype-specific primers from the envelope non-structural protein junction gene sequence [24]. CYD dengue full-length viral RNA standards included in each run allowed the results to be expressed as a concentration of $\log_{10}$ Genome equivalents/mL.

Please cite this article in press as: Dayan GH, et al. Safety and immunogenicity of three tetravalent dengue vaccine formulations in healthy adults in the USA. Vaccine (2013), http://dx.doi.org/10.1016/j.vaccine.2013.08.088