EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 October 2018
EMA/791273/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Dengvaxia

Common name: dengue tetravalent vaccine (live, attenuated)

Procedure No. EMEA/H/C/004171/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

1/dil	reciprocal of dilution
Ab	antibody
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AND	acute neurotropic disease
AP	Pacific
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical Classification
AVD	acute viscerotropic disease
CCID50	cell-culture infectious dose 50%
CDP	Clinical Development Program
CI	Confidence interval
CoP	Correlate of protection
CoR	Correlate of risk
CYD	Chimeric Yellow fever Dengue
DF	dengue fever
DHF	dengue hemorrhagic fever
DP	drug product
DS	drug substance
DSS	dengue shock syndrome
E	envelope
EDC	Estimated Date of Conception
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FASE	full analysis set for efficacy
FASI	full analysis set for immunogenicity
FDA	Food and Drug Administration
FV	flavivirus
GMO	genetically modified organism

GMT	Geometric mean titer
GMTR	Geometric mean of titer ratio
HSA	Human Serum Albumin
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
LatAm	Latin America
LMP	last menstrual period
MedDRA	Medical Dictionary for Regulatory Activities
mFASE	modified full analysis set for efficacy
MMR	measles/mumps/rubella
MN	microneutralization
NS1	non-structural 1
PoC	proof of concept
PPSE	per-protocol analysis set for efficacy
prM	Pre-membrane
PRNT	plaque reduction neutralization test
PT	Preferred term
RDT	Rapid Diagnostic Test
RMP	Risk Management Plan
RT-PCR	reverse transcription-polymerase chain reaction
SAE	Serious adverse event
SC	subcutaneous
SEA	South-East Africa
SOC	System Organ Class
SVCD	severe virologically-confirmed dengue
VCD	virologically-confirmed dengue
VE	vaccine efficacy
WBC	white blood cells
WHO	World Health Organization
wt	wild type
YF	yellow fever

1. Background information on the procedure

1.1. Submission of the dossier

The Applicant Sanofi Pasteur SA submitted on 2 March 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Dengvaxia, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The Applicant applied for the following indication

"Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 60 years of age living in endemic areas. The use of Dengvaxia should be based on official recommendations."

The Applicant has changed to Sanofi Pasteur during the procedure at Day 181.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0174/2015 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0174/2015 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the Applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The Applicant requested the active substance contained in Dengvaxia dengue tetravalent vaccine (live, attenuated), consisting of chimeric yellow fever dengue virus serotypes 1, 2, 3 and 4 (live, attenuated), to be considered as a new active substance, as the Applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific advice

The Applicant received Scientific advice from the CHMP:

Scientific advice	date	Area
EMA/CHMP/SAWP/430177/2013	25 July 2013	The scientific advice pertained to clinical aspects of the dossier
EMA/CHMP/SAWP/827728/2015	26 February 2015	The scientific advice pertained to quality aspects of the dossier
EMA/CHMP/SAWP/374073/2015	25 June 2015	The scientific advice pertained to clinical aspects of the dossier

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Bart Van der Schueren Co-Rapporteur: Sol Ruiz

The application was received by the EMA on	2 March 2016
The procedure started on	24 March 2016
The Rapporteur's first Assessment Report was circulated to all CHMP members on	15 June 2016
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	10 June 2016
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	24 June 2016
The CHMP agreed on the consolidated List of Questions to be sent to the Applicant during the meeting on	21 July 2016
The Applicant submitted the responses to the CHMP consolidated List of Questions on	17 January 2017
The following GCP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
A GCP inspection at 6 sites (5 investigator sites in Vietnam and one investigator site in Indonesia) has been conducted between August and November 2016. The outcome of the inspection carried out was issued on	23 December 2016
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	2 March 2017
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	9 March 2017

The CHMP agreed on a list of outstanding issues in writing to be sent to the Applicant on	23 March 2017
The Applicant submitted the responses to the CHMP List of Outstanding Issues on	26 March 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	11 April 2018
The CHMP agreed on a second list of outstanding issues in writing to be sent to the Applicant on	26 April 2018
SAG was convened to address questions raised by the CHMP on 26 April 2018 The CHMP considered the views of the SAG as presented in the minutes of this meeting.	30 April 2018
The Applicant submitted the responses to the second CHMP List of Outstanding Issues on	29 June 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	6 September 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Dengvaxia on	18 October 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Dengue disease is a mosquito-borne viral disease. The four dengue virus serotypes (DENV 1, 2, 3, and 4) are transmitted by mosquitoes of the Aedes family, primarily *Aedes aegypti*. The infection is mostly asymptomatic or causing mild, flu like illness but it can develop into a potentially lethal complication called severe dengue, including dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Infection by each serotype is considered to induce serotype-specific lifetime immunity.

Dengue disease is a major public health concern in more than 100 countries, with the four dengue virus serotypes found in tropical and sub-tropical regions, including some European regions.

The global incidence of dengue has grown dramatically in recent decades and half of the world's population is now considered at risk of infection by the dengue viruses. Worldwide, an estimated 390 million dengue infections occur every year, of which around 100 million are associated with clinical manifestation of dengue. Around 2 million cases evolve to severe dengue, and around 20,000 cases would result in death. Severe dengue is a leading cause of serious illness and death among children in endemic countries.

2.1.2. Epidemiology and risk factors

Risk factors

Epidemiologic studies have identified young age, female sex, high body-mass index, virus strain, and genetic variants of the human major-histocompatibility-complex class I–related sequence B and phospholipase C epsilon 1 genes as risk factors for severe dengue. Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock. Host genetic determinants might influence the clinical outcome of infection, though most studies have been unable to adequately address this issue. Secondary infection, in the form of two sequential infections by different serotypes, is also an epidemiologic risk factor for severe disease. Mechanistically, increased risk in secondary infection is thought to be linked to antibody-dependent enhancement of virus infection in Fc receptor–bearing cells and the generation of a large infected cell mass *in vivo*, which might promote capillary permeability.

Chronic disease (bronchial asthma, sickle cell anaemia and diabetes mellitus) and ethnicity may represent additional individual risk factors that determine the severity of disease. Studies in the American region show the rates of severe dengue to be lower in individuals of African ancestry than those in other ethnic groups.

Epidemiology of Dengue virus

The terms 'endemicity' and 'hyperendemicity' are used to indicate the simultaneous circulation of one or several Dengue virus serotypes, respectively. Dengue epidemiology varies across regions and seasons, meaning that simultaneous exposure to all 4 DENV serotypes is highly unlikely in a natural setting. An endemic region is defined as a region where cases are present over the majority of time during the year. This means that transmission is constantly ongoing. In contrast, an epidemic region is a region where cases are only present during a short period of time. Yearly epidemics can happen, or an epidemic can happen over several years.

Endemicity is therefore not really linked to seroprevalence. During an epidemic 70% of the population could be infected, hence a seroprevalence of 70% could be reached for that circulating serotype, but when there is no transmission in the rest of the year(s), it is not considered an endemic region. Dengue epidemiology is dynamic in serotype prevalence. The prevalence of each serotype fluctuates over time, as does the genetic diversity within each serotype. The four dengue virus serotypes are genetically diverse and share limited identity (around 60-75%) at the aminoacid level. Genetic variations between serotypes and clades may be important determinants of differential viral fitness, virulence and epidemic potential.

Endemicity depends on local transmission possibilities, hence on the distribution of the *Aedes* vector. *Aedes aegypti* is the best vector for dengue, but it is unlikely that this vector will be established in Europe; hence the risk that continuous transmission will be ongoing due to this vector is very low. *Aedes albopictus* is a less good vector, but it is likely that it will spread more widely over Southern Europe. Hence when the virus comes into a locality, and when there are vectors and susceptible persons within a certain population density, there is possibility for transmission and eventually endemicity (if the mosquito survives European winters).

The global burden of dengue disease is mainly in inter-tropical areas and most EU Outermost Regions and Overseas Countries and Territories are in dengue endemic regions. Sustained transmission of dengue fever does not naturally occur in continental Europe, though sporadic autochthonous dengue cases had been reported e.g. in Croatia in 2010 and in France in several recent years. EU dengue endemic areas include tropical Latin America, the Caribbean and the Indian & Pacific Oceans and epidemiology varies by region. In the Caribbeans and Latin America, a high level transmission and endemicity is demonstrated by incidence rates during epidemics, seroprevalence and 4-serotype circulation. The reported seroprevalence among adults ≥18 years-old was >80%, and >90% in certain settings. In the EU territories outside the Americas, limited data suggest lower endemicity. Seroprevalence among adults is estimated <50% in the putermost regions in the Indian Ocean (La Reunion, Mayotte). La Reunion showed unusual persistent circulation of dengue in 2017-2018, perhaps indicating a changing epidemiology. Very few surveys on Dengue seroprevalence in continental Europe are done, given the lack of endemicity. In Croatia, seroprevalence rates was calculated by county and varied between 0 and 2.21%[1].

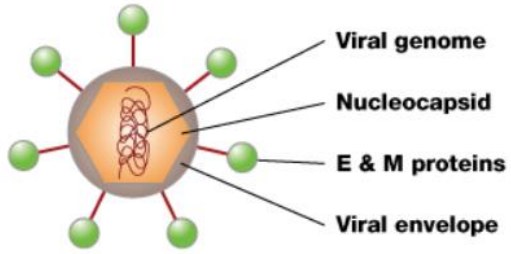
In endemic areas, the entire population is at risk of dengue infection. The disease affects all age groups. The age distribution of infected individuals varies between countries and no clear pattern of populations at risk has been identified. For example, incidence rates were highest in adults in Mexico, Malaysia, and in the French Caribbean, highest in adolescents in Brazil and Thailand, and highest in children in the Philippines and Colombia. Additionally, the population at highest risk can shift over time, as was observed in Colombia and Thailand over the last decade.

2.1.3. Aetiology

The aetiological agent of Dengue fever is dengue virus (DENV), which is a mosquito-borne single positive-stranded RNA virus of the family *Flaviviridae*, genus *Flavivirus*. The Dengue virus has a roughly spherical shape. Inside the virus is the nucleocapsid, which is made of the viral genome and C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

[1] Pem-Novosel I, Vilibic-Cavlek T, Gjenero-Margan I, Kaic B, Babic-Erceg A, Merdic E, et al. Dengue virus infection in Croatia: seroprevalence and entomological study. New Microbiol 2015 Jan;38(1):97-100.

Figure 1: Dengue virus structure.

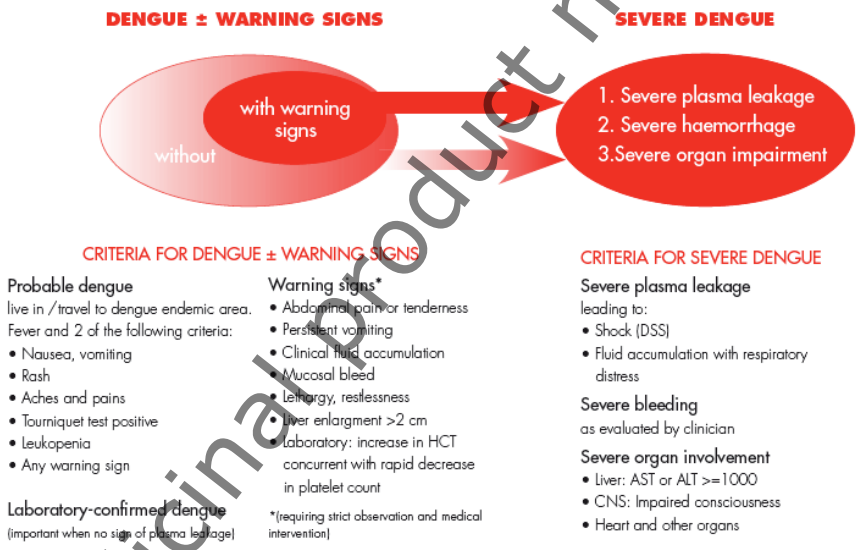


2.1.4. Clinical presentation, diagnosis

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following a self-limiting non-severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without haemorrhage. Intravenous rehydration is the therapy of choice; this intervention can reduce the case fatality rate to less than 1% of severe cases. The group progressing from non-severe to severe disease is difficult to define, but this is an important concern since appropriate treatment may prevent these patients from developing more severe clinical conditions.

Despite discussions regarding the classification of dengue cases, classification into DF/DHF/DSS continues to be widely used. Symptomatic dengue virus infections are grouped into three categories: undifferentiated fever, dengue fever (DF) and dengue haemorrhagic fever (DHF). DHF is further classified into four severity grades, with grades III and IV being defined as dengue shock syndrome (DSS).

Figure 2: Suggested dengue case classification and levels of severity



Primary dengue virus infection is thought to provide lifelong protection against the infecting serotype and transient cross-protection against heterologous serotypes. Dengue haemorrhagic fever and dengue shock syndrome occur mostly in individuals during secondary dengue virus infection with a different serotype. The underlying mechanism, referred to as antibody-mediated enhancement of dengue, seems to be related with the presence of suboptimal neutralizing heterotypic antibodies, and may also be related to the presence of memory T cells with low affinity for the present infecting virus but high affinity for previous infecting serotype(s). It is widely recognised that, since pre-existing immunity to dengue can

increase the risk of dengue haemorrhagic fever, a successful vaccine should simultaneously generate long-lasting protective immunity against all four dengue serotypes viruses.

Cross-immunity between Flaviviridae

There is no known cross-immunity between Flaviviridae. There is no evidence that if someone had yellow fever disease, this person would be less susceptible for Dengue infection. There are also no indications that YF vaccination would trigger clinical immunity to Dengue. However, a cross reaction of IgG with dengue diagnostic IgG test is generally observed.

No interference of Dengue and Zika severity has been observed in a retrospective study in French Polynesia. In that study, no relationship between history of Dengue infection and severity of Zika virus infection (Guillain-Barré syndrome) was found.

Specificity of Flavivirus diagnostic tests

Routine laboratory diagnosis of dengue infections is based on one or more of the following: the detection of dengue virus–specific antibodies (IgM), isolation of the virus, detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR), or detection of viral protein NS1 antigen by enzyme-linked immunosorbent assay (ELISA). After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis. The diagnosis of dengue falls into 2 stages: Stage I, the acute fever period lasting a few days when viremia may be detected; and Stage II, the early post-febrile period lasting a few weeks when IgM and IgG antibodies are increased.

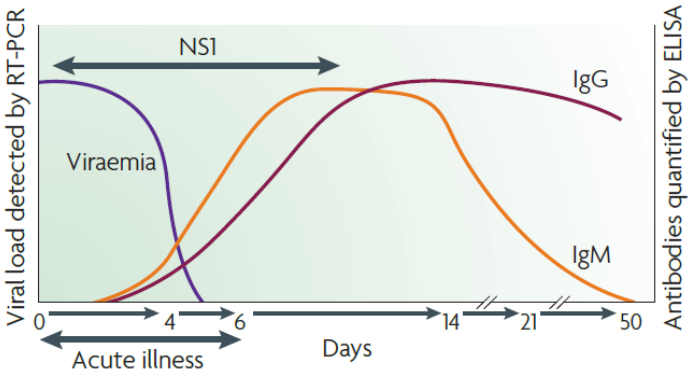
Cross-reactions on diagnostic tests are observed among flaviviruses. Dengue ELISA tests are cross-reacting with other flavivirus infections (specificity of 77-98%, sensitivity of 21-99% depending on RDT test used[2]). The Plaque Reduction Neutralisation test (PRNT) is the most specific one and has been largely studied in preparation of the vaccine trials[3].

Cross-reactivity of an antibody-based Dengue test when someone has a Zika infection is a described problem[4]. RT-PCR is 98% sensitive and 98% specific depending on time of sampling after onset of disease[5]. NS1 ELISA has a specificity of 95-100%. In contrast, the Zika ELISA antibody test is very specific and very sensitive (based on current knowledge) and does not cross-react importantly. If someone has Dengue or another Flavivirus infection, the Zika ELISA test does not mark falsely positive[6].

The time point at which blood sampling is performed for dengue and other Flavivirus diagnostics is important as illustrated in Figure 3.

[2] Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, et al. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Emerg Infect Dis 2009 Mar;15(3):436-40.
[3] Thomas SJ, Rothman AL. Trials and tribulations on the path to developing a dengue vaccine. Vaccine 2015 Nov 27;33 Suppl 4:D24-D31.
[4] Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. N Engl J Med 2016 Apr 21;374(16):1552-63.
[5] Wu et al., AJTMH, 2008
[6] Huzly D, Hanselmann I, Schmidt-Chanasit J, Panning M. High specificity of a novel Zika virus ELISA in European patients after exposure to different flaviviruses. Euro Surveill 2016 Apr 21;21(16).

Figure 3: Dengue virus, antigen and antibody responses used in diagnosis. Ig, immunoglobulin; NS, non-structural.



2.1.5. Management

There is no specific treatment against dengue disease and, at the date of Dengvaxia initial application (March 2016), there was no licensed vaccine to prevent dengue infection or diseaseso prevention of dengue rested upon the vector control programs and personal protection. These are of limited efficacy, difficult to enforce and expensive to maintain.

Vaccination remains the best approach to control this disease and there is need of a vaccine especially for people living in endemic countries also in the EU. To this day (October 2018), the CYD dengue vaccine has been licensed in Thailand, Singapore, Malaysia, Cambodia, Australia, Bolivia, Argentina, Honduras, Mexico, the Philippines, Brazil, El Salvador, Paraguay, Costa Rica, Indonesia, Peru, Venezuela, Guatemala, Bangladesh, Myanmar and Dominican Republic under the name of Dengvaxia. The Marketing Authorisation was suspended for one year in The Philippines as of January 2018.

About the product

Dengvaxia is a prophylactic, tetravalent, live attenuated viral vaccine against DENV. Throughout the document the term CYD dengue vaccine will be used to identify Dengvaxia. The active substances contained in the CYD dengue vaccine are 4 live attenuated chimeric yellow fever dengue viruses (serotypes 1, 2, 3, and 4). Each monovalent CYD dengue virus was obtained separately via recombinant deoxyribonucleic acid (DNA) technology. The CYD dengue viruses were constructed by replacing the gene encoding the pre-membrane (prM) and envelope (E) proteins in the attenuated yellow fever (YF) 17D virus genome by the corresponding genes of the 4 wild type dengue serotypes 1, 2, 3 and 4. The final formulation contains ~5 log10 cell-culture infectious dose 50% ($CCID_{50}$) of each live attenuated, chimeric dengue serotype 1, 2, 3 and 4 viruses.

The CYD dengue vaccine, initially developed as liquid batches, is a sterile and freeze-dried product to be reconstituted before injection with either a sterile solution of 0.4% sodium chloride for the single-dose presentation or a sterile solution of 0.9% sodium chloride for the multidose (5 doses) presentation. After reconstitution, one dose (0.5 mL) is to be administered by the subcutaneous (SC) route. The vaccine is presented in a single-dose vial or in a 5-dose multidose vial.

Dengvaxia initially proposed indication was the following: prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 60 years of age living in endemic areas. The use of Dengvaxia should be based on official recommendations.

2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) is presented as a powder and solvent for suspension for injection in vial and pre-filled syringe/vial (for the solvent) containing 4.5 - 6.0 log^{10} CCID50 (50% Cell Culture Infectious Dose)/dose of each of the four serotypes as follows: Live, attenuated, chimeric dengue virus, serotype 1 / Live, attenuated, chimeric dengue virus, serotype 2 / Live, attenuated, chimeric dengue virus, serotype 3 / Live, attenuated, chimeric dengue virus, serotype 4.

Other ingredients are as follows: (for the powder) essential amino acids including phenylalanine, non-essential amino acids, arginine hydrochloride, sucrose, trehalose dihydrate, sorbitol (E420), trometamol and urea; (for the solvent for reconstitution) sodium chloride and water for injections. The vaccine does not contain a preservative.

The product is available as a single-dose and five-dose formulation and presented in a vial containing the powder and a pre-filled syringe (single-dose) or a second vial (5-dose) containing the solvent for reconstitution.

For the single-dose formulation, the powder (1 dose) is presented in a Type I glass vial with a stopper (halobutyl) and a flip off cap (aluminum, polypropylene) and a pre-filled syringe (Type-I glass) containing 0.5 mL of solvent with a plunger stopper (halobutyl) and a tip cap (elastomer) with or without 2 separate needles.

For the five-dose formulation, the powder (5 doses) is presented in a type-I glass vial with a stopper (halobutyl) and a flip-off cap (aluminum, polypropylene) and a second vial (Type-I glass) containing 2.5 mL of solvent with a stopper (halobutyl) and a flip-off cap (aluminum, polypropylene).

2.2.2. Active Substance

General information

Dengvaxia is a tetravalent, live attenuated dengue viral vaccine based on a chimaeric yellow fever virus-dengue virus (CYD). Each CYD dengue virus serotype was obtained separately from parental yellow fever 17D virus (YF-17D) and wild-type (wt) dengue viruses 1-4 via recombinant DNA technology by replacing the sequence encoding the pre-membrane (prM) and envelope (E) proteins in the parental yellow fever 17D (YF-17D) virus genome by those encoding for the homologous sequences of the four wt dengue serotypes 1, 2, 3 and 4. No additional sequences are added.

The exchange of the prM and E coding sequences from the YF-17D virus for those of the 4 dengue viruses results in the production of 4 CYD dengue virions (one for each serotype), expressing the envelope protein of each wt dengue virus strain at their surface. The envelope protein(s) determine the cellular tropism, while viral replication in these cells is determined mainly by the YF-17D virus replication engine. The immunising antigens are the prM and E proteins from the wt dengue viruses. The CYD dengue viruses 1-4 do not contain genetic information for the prM and E proteins of the YF-17D virus as these sequences have been replaced by those of the corresponding wt dengue viruses.

The YF-17D virus and the wt dengue virus serotypes 1-4 are members of the Flaviviridae family. The structure of CYD virions and their mode of replication in infected cells are the same as other flaviviruses. The flavivirus particles have a diameter of approximately 50 nm and contain a positive-sense, single-stranded RNA genome. The RNA genome encodes the structural and the non-structural proteins in a single open reading frame. The 5' end of the viral genome contains three structural proteins: the capsid

(C) protein, the pre-membrane (prM) and envelope (E) proteins. The 3' end of the viral genome contains seven non-structural (NS) proteins that consist of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The E protein is the primary surface structural protein. It contains the antigenic determinants that define protective immunity (neutralising epitopes) and is essential for membrane fusion and binding to cellular receptors. The prM protein is known to be important for morphogenesis of viral particles. It facilitates proper folding of E and also functions to protect the E protein dimer from premature conformational rearrangement during passage of progeny viral particles towards the cell surface through acidic secretory compartments.

The Applicant has provided detailed information on the structure and general properties of the 4 chimeric yellow fever/dengue (CYD) virus serotypes.

Manufacture, characterisation and process controls

Manufacture and quality control of Dengvaxia active substances (ASs) takes place at Sanofi Pasteur NVL (31-33 quai Armand Barbès, 69250 Neuville-sur-Saône, France) and Sanofi Pasteur MLE (1541 avenue Marcel Merieux, 69280 Marcy l'Etoile,France).

Valid GMP certificates for these sites have been presented.

Description of the manufacturing process and process controls

The manufacture of CYD dengue active substance is the same for the four virus serotypes and is divided into 5 major manufacturing process stages: (1) Cell culture, (2) Viral culture and clarification, (3) Purification, (4) Concentration and diafiltration and (5) Stabilisation, filling and storage.

One batch of Dengvaxia active substance is obtained from one single batch of cell culture and viral infection with one serotype followed by a purification process. The batch number is a unique and non-descriptive sequence of characters that is automatically assigned by a manufacturing planning system.

Manufacturing begins with thawing of serum-free Vero working cell bank (WCB). The Vero cells are cultivated and expanded. After cell expansion the cultures are inoculated with CYD virus and propagated. The viral culture is harvested and clarified. Clarified Harvest is purified by chromatography and further processed without a holding step. The Purified Harvest is concentrated through diafiltration. The Concentrated Harvest is then mixed with stabilizer solution filtered and filled in storage bags and stored at ≤-70°C to obtain the active substance.

In-process controls and critical parameters have been provided together with their acceptance criteria.

Total protein content is determined to monitor and control consistency of the Concentrated Harvest. Since host cell protein constitutes more than 99% of the total protein in the active substance, the total protein is indicative of the host cell protein. As such provided proper limits are applied for total protein, it is acceptable not to include host cell protein in the AS specifications. As long as no alert limits have been set for total protein, the Applicant is recommended to communicate any out-of-trend results for total protein content to the Agency. As soon as sufficient data are available for all serotypes (post-approval), the Applicant should set proper (serotype-specific) alert limits for total protein.

The container closure system for the active substance is a sterile storage bag.

The container closure system is in compliance with Ph. Eur. 3.2.2.1 tests ("Plastic Containers for Aqueous Solutions for Parenteral Infusion"). Routine sterility testing is performed following ANSI/AAMI/ISO 11137 guidelines. The suitability of the container closure system for storage of the AS has been demonstrated.

For transportation, storage bags of each serotype of CYD dengue active substance are placed within a container, as a secondary packaging. The container is maintained frozen in a box with dry ice so that the CYD dengue AS is maintained at ≤-70°C during transportation.

The manufacturing process of CYD Dengue AS has been adequately described with detailed and clear flowcharts.

All clinical AS lots were produced at the MLE site (Marcy l'Etoile). Comparability between the commercial NVL site and the MLE site has been demonstrated (see manufacturing process development).

Control of materials

The list of raw materials used in the manufacture of CYD dengue active substance is described in detail.

No materials of animal or human origin have been used in the production of master seed lot (MSL) and master cell bank (MCB) and onwards. Nevertheless, batches have been tested for the presence of viral and non-viral adventitious agents, demonstrating the absence of them.

The generation of the MCB and WCB is well described. The number of passages is taken into consideration. An extensive characterisation of MCB and WCB was performed.

A description of the chimeric construct (YF 17D virus and wild type dengue viruses 1-4) is provided and it is considered adequate. The generation of the MSL and working seed lot (WSL) in Vero cells is well described.

Long-term stability studies were performed on WSL.

It is agreed that the MCB, WCB, MSL and WSL are suitable for use in pharmaceutical production of CYD Dengue active substance.

Process validation

The active substance manufacturing process has been appropriately validated.

The validation studies performed for the manufacturing process show that all critical process parameters (CPPs) comply with their operating ranges or limits, and are monitored at their target values. All results for release and IPC tests meet the acceptance criteria and all characterisation and additional test results are consistent and show no atypical values. The validation data also demonstrate that process performance with regards to virus concentration yields and impurities removal is reproducible and that any process-related adventitious agent contamination is well controlled.

Manufacturing process development

The Applicant manufactured all AS batches from phase I to phase III at Marcy l'Etoile site (MLE, France). The AS manufacturing process was transferred and scaled-up fromMLE site to NVL site to ensure a sustainable vaccine supply for vaccination at a large scale. Some process adaptations were implemented at NVL to allow for a scale up of the downstream manufacturing process.

Comparability studies were performed and showed that the quality of CYD dengue virus manufactured by the commercial process proposed by the Applicant is highly comparable to the material obtained from earlier processes (including batches tested during non-clinical and clinical studies).

The Applicant has provided a justification and risk assessment to demonstrate that the commercial batches from the NVL site can be considered as comparable to the clinical batches in terms of their critical quality attributes. The Applicant has further committed to analyse virion maturation for at least 3 commercial batches from the NVL site for all 4 serotypes in order to further demonstrate that virion

maturation is consistent and in line with the results obtained for the clinical batches and the first tested NVL batches.

Characterisation

The CYD dengue virus particles are considered undistinguishable from native dengue virus particles and are expected to display the same surface arrangement that is described for dengue and several other flaviviruses.

The structure/function of CYD dengue viral proteins, as well as their resulting safety and immunogenicity profile are determined by the CYD dengue virus genome sequence.

Genetic stability is evaluated by genetic sequencing of viral particles and comparing the RNA genome sequences of the viral strains obtained at the active substance stage and at further passages beyond with the sequences issued from the Pre-Master Seed Lots (PMSL). In addition, genetic stability is assessed indirectly via a plaque size test and a suckling mice neurovirulence test. The mice/suckling mice neurovirulence test and the plaque size assay are historical phenotypic tests applied on flaviviruses.

A potency assay has been established by the Applicant. This assay is based on measuring the infectivity titre by cell culture infectious dose (CCID50) and allows identifying the virus serotype. The Applicant has committed to establish a method to analyse virion maturation as characterisation test post-authorisation in order to assess future changes that may have an impact on the virion composition/maturation. The Applicant has also committed to characterize virion maturation in case of qualification of new virus working seeds and will demonstrated for each new WSL that the corresponding AS batches show consistent virus maturation that is in line with batches derived from previous WSLs.

Attenuation of viscerotropism and neurotropism in CYD dengue viruses was demonstrated for each serotype in *in vitro* and preclinical *in vivo* experiments.

The product purity is controlled by implementing different viral and non-viral adventitious agent detection tests at the appropriate stage of production of the active substance. These tests are carried out as release tests.

Process controls

The CYD dengue vaccine candidate is a vaccine for which attenuation basis and characteristics are well defined and have been carefully assessed. The Applicant has demonstrated satisfactory stability, safety and immunogenicity of the vaccine candidate in an exhaustive set of *in vitro* and *in vivo* preclinical tests. All results from the preclinical studies are consistent with the stability, safety and immunogenicity of the CYD dengue vaccine candidate.

The impurities present for CYD Dengue active substances are appropriately characterised.

Specification

The Applicant has assembled an appropriate set of specification tests and acceptance criteria to adequately control the release of CYD Dengue virus active substance as well as the end of shelf life.

The release specification and the defined acceptance criteria are well justified and based on current regulations such as Ph. Eur. monograph 0153 and Ph. Eur. 2.6.16, WHO Technical Report Series, No. 979 Annex 2, Guidance for Industry (FDA, CBER, 2010), and EMA/CHMP/VWP/141697/2009.

The end of shelf-life specification and the defined acceptance criteria are based on ICHQ5C. The setting of the specifications was also informed by data obtained during the development and stability studies.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

A potency assay is performed at release. This assay is based on measuring the infectivity titre by cell culture infectious dose ($CCID_{50}$) and allows identifying the virus serotype (see above).

Batch analysis

Release testing data have been presented.

All batches conform to the specifications, supporting the consistency of the CYD Dengue viruses manufacturing process.

Reference materials

The only reference material used in the control of the active substance relates to the residual Vero DNA content assay. The reference standard for the residual Vero DNA content assay and its qualification are described.

Stability

The proposed shelf life for storage of the active substance is 42 months stored at ≤-70°C.

Stability studies have been performed for CYD Dengue AS using both, long term storage conditions (≤ -70°C) and accelerated conditions (+5 ± 3°C). Stability data of 42 months from three batches per serotype stored at ≤ -70°C and of 30 days for three batches per serotype stored at +5 ± 3°C have been provided.

All stability data provided meet the pre-set requirements. Based on the available stability data, the long-term stability studies up to 42 months performed on CYD dengue active substance manufactured at MLE and NVL sites support a shelf-life of 42 months when stored at ≤-70°C. In addition, the accelerated stability studies up to 30 days at +5°C ± 3°C performed on AS from both manufacturing sites demonstrated a similar decrease of virus concentration below 1 log for each serotype and within the ≤-70°C specification, supporting a possible cold chain break.

The Applicant commits to perform stability studies on the AS in the context of the annual stability program, and to inform the Competent Authority in the event of unexpected issues.

Overall, the stability data and program presented by the Applicant is considered adequate.

2.2.3. Finished Medicinal Product

Description of the product

Dengvaxia is formulated as a powder and solvent for suspension for injection administered by subcutaneous route. The finished product contains 4.5 - 6.0 $\log^{10}$ CCID50 (50% Cell Culture Infectious Dose)/dose of each of the four serotypes. It is a white homogeneous freeze-dried product filled in a glass vial.

Other ingredients are (for the powder) essential amino acids including phenylalanine, non-essential amino acids, arginine hydrochloride, sucrose, trehalose dihydrate, sorbitol (E420), trometamol and urea (all stabilisers) and (for the solvent for reconstitution) sodium chloride and water for injections.

The solvent used for reconstitution is a 0.4% sodium chloride solution for the mono-dose and 0.9% sodium chloride solution for the five-dose vaccine. The reconstituted product is a colourless limpid liquid with possible presence of white to translucent particles of endogenous nature. One dose consists of a volume of 0.5 mL after reconstitution with the solvent.

For the single-dose formulation, the powder (1 dose) is presented in a Type I glass vial with a stopper (halobutyl) and a flip off cap (aluminum, polypropylene) and a pre-filled syringe (Type-I glass) containing 0.5 mL of solvent with a plunger stopper (halobutyl) and a tip cap (elastomer) with or without 2 separate needles.

For the five-dose formulation, the powder (5 doses) is presented in a type-I glass vial with a stopper (halobutyl) and a flip-off cap (aluminum, polypropylene) and a second vial (Type-I glass) containing 2.5 mL of solvent with a stopper (halobutyl) and a flip-off cap (aluminum, polypropylene).

The compatibility between the CYD dengue viruses and the chosen excipients has been demonstrated by the stability studies performed under normal and accelerated conditions. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph.Eur. standards when an applicable monograph exists. There are no novel excipients used in the finished product.

For NaCl 0.4%, the Applicant detailed the validations and qualifications of the blending step as well as the validations linked to the major changes arisen in the recent years on the manufacturing process development: terminal sterilization by heating and the inclusion of an additional manufacturing site.

For NaCl 0.9%, the Applicant detailed the validation of the Final Bulk Product manufacturing process, the terminal sterilisation process development and the determination of the hold period for the filled vials prior to terminal sterilisation.

In addition, the Applicant demonstrated the compatibility between the solvents and the chosen container closure systems for final bulk product and filled product using a series of physicochemical and biological tests as well as available stability studies.

Pharmaceutical development

The Applicant provided adequate information of the formulation development from early stage clinical development to phase III.

The Applicant provided a comprehensive description of batches manufactured during pharmaceutical development as well as changes in the manufacturing process from Phase I to Phase III. The Applicant presented a comprehensive description of Phase III and commercial manufacturing process, including important in-process controls as well as qualification/validation of the different process steps.

The Applicant also performed a comparability exercise to demonstrate that quality attributes are highly similar across the process development phases (I, II, III and commercial). As all batch analysis results were compliant to the specifications, the Applicant concluded that the formulations are comparable and an adverse impact on safety or efficacy profiles can be excluded.

Finally, the compatibility between CYD Dengue vaccine and the container closure system was demonstrated using physicochemical tests, cytotoxic studies, and stability studies under normal and accelerated conditions.

The information presented in relation to pharmaceutical development is considered adequate.

Manufacture of the product and process controls

The manufacture of Dengvaxia freeze-dried finished product takes place at Sanofi Pasteur VDR (Parc Industriel d'Incarville, 27100 Val de Reuil, France). The sites responsible for batch release of the finished product are Sanofi Pasteur NVL and Sanofi Pasteur VDR.

The description of the manufacturing processes for Dengvaxia finished product and freeze-dried product is considered adequate. Each CYD dengue virus serotype is thawed and transferred to a mixing tank, which contains the FBP stabilising solution. In the mixing tank the FBP stabilising solution is then added up to the final batch size volume and stirred. Then, the FBP is sterilized by filtration, filled into vials, partially stoppered, freeze-dried, capped, crimped and at the end, visually inspected.

The manufacturing processes of the solvents (Sodium chloride solution, NaCl 0.4% and 0.9%) involved the following steps: Water for Injections (WFI) is introduced into a sterile stainless steel tank and sodium chloride is introduced into the tank under agitation until complete dissolution. The tank is then filled up to production batch size with WFI and is filtrated through a 0.2 µm filter to obtain the FBP. The FBP is then filtered at room temperature through a 0.2 µm filter and filled into the final container, stoppered, sterilized and final inspected to obtain the filled product.

The in-process controls and acceptance criteria are well defined. Validation of critical steps has also been addressed appropriately.

The Applicant also described the storage and transportation conditions under controlled temperature. The critical process parameters, in-process controls, and acceptance criteria are well defined. Validation of critical steps (i.e. blending, sterile filtration, filling and freeze-drying) has also been performed on 3 consecutive batches. In addition to this initial validation, the Applicant implemented on-line sterile filtration at the filling step.

Overall, the information provided by the Applicant in the manufacture section is considered extensive and adequate.

Product specification

The CYD dengue vaccine is appropriately controlled by release and end of shelf-life specifications. These include tests for physicochemical properties, virus concentration and identity, endotoxins and sterility.

The specifications used to control the freeze-dried product during annual stability programs for both single-dose and multi-dose additionally consider stability indicating tests such as the container closure integrity.

Analytical methods

Most tests used for release are in compliance with pharmacopoeial methods and therefore analytical validation data are not provided. This is considered acceptable.

For analytical procedures not described in pharmacopoeias description of the analytical methods and relevant analytical validation results are provided.

As for the AS specifications, a potency assay is performed at release for the FP. This assay is based on measuring the infectivity titre by cell culture infectious dose (CCID50) and allows identifying the virus serotype.

It can be concluded that the analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines.

Batch analysis

Batches of CYD dengue FBP and batches of freeze-dried product have been manufactured at industrial scale from AS produced in MLE and NVL sites.

No additional impurities are introduced during the manufacturing processes of the CYD dengue FBP and freeze dried product in addition to the ones described for the AS. The stated impurities have been studied in nonclinical and clinical studies.

All batches conformed to release specifications. The specifications are considered overall acceptable.

Reference materials

The only reference material used in the manufacture of the finished product relates to the bacterial endotoxin test. Bacterial endotoxin content is carried out by the chromogenic LAL kinetic method using a test kit. Each test kit is qualified using the Reference Standard Endotoxins (RSE) provided by the European Directorate for the Quality of Medicines (EDQM).

Stability of the product

The shelf life of Dengvaxia finished product is 3 years stored in a refrigerator (2°C - 8°C). The product should be stored in the outer carton in order to protect it from light.

After reconstitution with the solvent provided, Dengvaxia must be kept in a refrigerator (2°C to 8°C) and must be used within 6 hours.

To support the claimed shelf life the Applicant has provided data from stability studies.

Dengvaxia finished product

Stability studies were performed for the Dengvaxia freeze-dried finished product assessing the stability of FP derived from AS manufactured at Marcy l'Etoile site and at Neuville-sur-Saône site stored at +5°C ± 3°C for 36 months.

In addition the stability of the freeze-dried vaccine under accelerated storage conditions was assessed at +25°C ± 2°C over a period of 3 to 6 months and at +37°C ± 2°C over a period of 14 to 30 days to support possible cold chain break. Furthermore, the stability study on the reconstituted product was performed for each batch of CYD dengue vaccine over a period of 6 hours under normal storage conditions (+5°C ± 3°C).

Based on the available stability data for the freeze-dried finished product, the claimed shelf life of 36 months when stored at 2 - 8°C can be supported. The packaged product is photo stable.

Solvent

Stability studies were performed for final bulks and filled product for both the mono-dose (manufactured at the different sites) and the multi-dose presentation of the solvent. All results comply with the specifications.

Accelerated stability studies performed at +37°C ± 2°C or +40°C ± 2°C did not reveal any degradation pattern up to 6 months.

The stability studies performed on Filled Product (both NaCl 0.4% and NaCl 0.9%) support a shelf-life of 60 months when stored at real time storage condition.

The Applicant commits to complete the on-going stability studies and to inform the Competent Authority in the event of unexpected issues.

Overall, the stability data and program presented by the Applicant is considered adequate.

The Applicant commits to complete the on-going stability study on the solvent unidose, to perform stability studies on the finished product in the context of the annual stability program.

Comparability exercise for finished medicinal product

Not applicable.

Adventitious agents

The Applicant has developed a serum-free manufacturing process and eliminated bovine serum and porcine trypsin from the manufacturing process as well as for the manufacture ofviral seed lots and cell banks (i.e. from MCB and MSL). The safety of the CYD dengue vaccine with regard to viral and non-viral contamination has been assessed throuth three different approaches:

There is no dedicated viral inactivation or clearance step in the CYD dengue manufacturing process as the vaccine is a live attenuated tetravalent vaccine.

- Selecting and testing cell lines and seed lots for the absence of adventitious agents according to regulatory requirements, use of appropriate environmental manufacturing conditions and application of good manufacturing practices throughout the production process.
- The CYD dengue vaccine is manufactured according to cGMP in classified areas to prevent microbial contamination of the product. Validated procedures are used for the cleaning, decontamination and sterilization of equipment and production areas. Medium, buffers and excipients used in the manufacturing process are 0.2 µm filtered before use and validated aseptic techniques are used in the filling of the FP.
- Testing the product at appropriate stages of the production process for the absence of adventitious agents. Adventitious agent specifications are based on regulatory requirements and on the evaluation of risks associated with raw materials used for production, cell substrate sensibility, and/or origin of the viral strain. Tests performed at the appropriate steps of production are detection tests for extraneous agents that comply with regulatory requirements

The Applicant has adequately demonstrated that the CYD dengue vaccine production is free from risk associated with the contamination of the CYD dengue vaccine by viral and non-viral (i.e. bacteria, Mycoplasma, TSE/BSE) agents. The product quality in relation to viral safety is ensured by testing the raw and starting materials and by monitoring relevant steps of the manufacturing process. In addition, the Applicant has implemented internal procedures based on cGMP principles to prevent contamination.

Based on all the information provided in this section, the quality of the CYD dengue vaccine is considered acceptable with regard to the risk of contamination by adventitious agents.

GMO

CYD dengue vaccine is a tetravalent, live attenuated viral vaccine. Each monovalent CYD virus was obtained via recombinant Deoxyribonucleic Acid (DNA) technology. The vaccine virus was constructed by replacing the sequences encoding the premembrane (prM) and envelope (E) structural proteins in yellow fever (YF) 17D virus genome by those encoding for the homologous sequences of the four wild dengue serotypes and is thus considered a GMO.

An environmental risk assessment was conducted during the initial MAA procedure as further detailed in section 2.3.5. (Ecotoxicity/environmental risk assessment).

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on the development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The process has been properly validated and is adequately controlled.

During the procedure three major objections have been raised related to detection of differences detected in accelerated stability studies, appearance of visible particles of exogenous nature after dissolution of lyophilised finished product and virion maturation of product manufactured at the Neuville site.

In relation to the major concern raised during the procedure on detected differences related to virus concentration and potency, the Applicant provided information on the lots of the finished product impacted. It could be confirmed that these lots were all produced from the same AS batches. These lots were rejected and the concern was considered to be resolved.

In relation to the major objection on the presence of visible particles of exogenous nature, the Applicant has presented a root-cause investigation to identify the nature and origin of the particles and filaments of exogenous nature that were observed. The Applicant also performed routine monitoring of exogenous particulates in FP batches and revealed no further observations of exogenous particles. The investigation of the exogenous particles and the measures proposed by the company are considered sufficient and it was concluded that the FP is sufficiently monitored to detect possible batches with exogenous particles that may have an impact on safety/efficacy.

A third major objection was raised during the procedure and related to virion maturation. Virion maturation is considered an important quality parameter and should be characterised at the level of the AS. The applicant has provided data on virion maturation from the commercial NVL site since they were not part of the initial submission. The results for virion maturation tend to be lower than clinical batches from MLE. The Applicant has provided a justification and risk assessment to demonstrate that the commercial batches are considered as comparable to the clinical batches in terms of their critical quality attributes. The observed difference is not expected to have any relevant impact on the immune response in vaccinees and it was considered unlikely that the observed difference in virion maturation could lead to an immune response that is significantly different from those observed in the clinical trials. Taken together, it was concluded that NVL lots can be considered comparable to the clinical batches in terms of their critical quality attributes.

Based on the review of the quality data and responses provided by the Applicant, all quality concerns have been resolved and the marketing authorisation application for Dengvaxia is considered approvable from a quality point of view with a number of recommendations as detailed below.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of Dengvaxia has been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. As long as no alert limits have been set for total protein, the Applicant is recommended to

communicate any out-of-trend results for total protein content to the Agency. Secondly, as soon as sufficient data are available for all serotypes, the Applicant should set proper (serotype-specific) alert limits for total protein, which should then be communicated to the Agency.

2. The Applicant is recommended to provide the data of the on-going stability study on the solvent unidose when this stability study has been completed.
3. The Applicant is recommended to analyse virion maturation for at least 3 commercial batches from the NVL site (using a suitable method) for all 4 serotypes in order to demonstrate that virion maturation is consistent and in line with (or can be considered as equivalent to) the results obtained for the clinical batches and the NVL demonstration batches.
4. The Applicant is recommended to include at least one method to analyse virion maturation as characterisation test in case of relevant future modifications in the AS process (i.e. changes which may have an impact on the virion composition/maturation).
5. The Applicant should characterise virion maturation (by at least one method) in case new virus working seeds will be qualified in the future. It should be demonstrated for each new WSL that the corresponding AS batches show consistent virus maturation that is in line with (or can be considered as equivalent to) batches derived from previous WSLs.

2.3. Non-clinical aspects

2.3.1. Introduction

The objectives of the nonclinical studies were to characterize the primary pharmacodynamic profile of the CYD dengue vaccine and to evaluate its safety. An overview of the non-clinical program is given in the tables below.

Table 1: Nonclinical Pharmacology Program

Objectives		Study number	Material used
Assessment of vaccine immunogenicity and viremia		SBi 1313-88 DEN010Mk DEN011Mk DEN012Mk DEN014Mk DEN016Mk	Research and Phase I lots Phase I and II lots Phase II lots Phase II lots Phase II lots Phase I, II and III lots
Assessment of immunogenicity and protection against wt viremia		SBi 1324-88 DEN020Mk/C3	Phase I lots Phase II and III lots
Assessment of the breadth of protection induced by CYD dengue vaccine	Evaluation of monkey sera	CN0901 CN1101	Sera from DEN014Mk Sera from DEN016Mk
	Evaluation of human sera	CN1102 CN1201	Sera from CYD28 phase II trial Sera from CYD13 phase II trial

Objectives		Study number	Material used
Assessment and mitigation of interferences between serotypes	Heterologous priming	DEN011Mk DEN014Mk	Phase II lots Phase II lots
	Administration at different anatomical sites	DEN012Mk DEN014Mk	Phase II lots Phase II lots
	Adaptation of virus content per dose	SBi 1324-88 DEN014Mk	Phase I lots Phase II lots
	Administration of a third dose	DEN014Mk	Phase II lots
Assessment of sensitization due to heterologous flavivirus pre-immunity		SBi 1324-88 DEN011Mk	Phase I lots Phase II lots

Table 2: Nonclinical Safety Program

Type of studies	Study number	Material used
Systemic and local toxicity		
Repeat-dose toxicity	RQH00006	Phase II lot
Developmental and reproductive toxicity		
Investigative studies for species selection	SP0056 IS0906 and SP0056 IS0907	Phase II lot
Preliminary DART for dose selection	SP0056 PS1002 and SP0056 PS1003	Phase III lot
Pivotal DART	SP0056 DV1013, SP0056 DV1014 and SP0056 DV1109	Phase III lot
Other toxicity		
Biodistribution and shedding	SP0056 BD1001	Phase III lot
Neurovirulence	T 100 001	Phase I lot

2.3.2. Pharmacology

Primary pharmacodynamic studies

Different species have been proposed to monitor the immunological and clinical outcomes of dengue infection. Immunocompromised mice such as A-129 or AG-129 mouse strains have been widely used in basic dengue research, as they can develop some symptomatic infection. However, the monkey was the species of choice for the Applicant's pharmacology studies. Monkeys do not develop symptomatic dengue disease upon dengue virus infection, but they do present viremia and develop subsequent immunity.

The 5 objectives of the Pharmacology studies were:

1. Assessment of Vaccine Immunogenicity and Viremia

2. Assessment of Immunogenicity and Protection against Wild-type Viremia
3. Assessment of the Breadth of Neutralization Induced by CYD Dengue Vaccine
4. Assessment and Mitigation of Interference between Serotypes
5. Assessment of Sensitization to Higher Infection/Viremia Due to Heterologous Flavivirus Pre-immunity.

The endpoints assessed upon subcutaneous immunization of monkeys were:

- Measurement of neutralizing antibodies to assess the immunogenicity of the vaccine, and
- Measurement of viremia, to measure attenuation of the CYD viruses and also to determine protective efficacy in animals previously vaccinated and then challenged with wild type viruses.

The objectives/endpoints and the studies performed to address them were considered adequate.

The studies performed showed that CYD dengue viruses produced minimal viremia titres in monkeys. In all cases titres were low (between 1 and 2.5 log10 Plaque Forming Unit (PFU)/mL) and did not exceed 7 days duration. These data indicated good attenuation of the vaccine strains. Moreover, the *in vivo* genetic stability of the CYD dengue viruses was also evaluated by sequencing individual virus plaques isolated from the last day of viremia when detectable, and viruses which contained mutations were evaluated in a suckling mouse neurovirulence test, showing that despite some mutations appearing in CYD-1 and CYD-3, all viruses isolated from monkeys were significantly less neurovirulent than the YF 17D vaccine.

As it was also found in humans, serotype 4 was the predominant CYD serotype in monkeys inducing measurable and reproducible viremia after tetravalent CYD vaccination. Regarding neutralizing antibodies, CYD-1 and CYD-4 were the dominant serotypes in monkeys, both when used as monovalents and when administered in tetravalent formulations. In humans however viruses CYD-2 and CYD-3 were more immunogenic.

Appropriate tests confirmed that CYD vaccine viruses reduced the viremia when monkeys were challenged with a highly virulent dengue virus. Moreover, as determined in *in vitro* cell culture assays, vaccine viruses induced an antibody response that protected against a wide range of different circulating strains (around 20 strains per serotype). Finally, and although these data have to be taken with caution due to the lack of knowledge of the underlying mechanism, the experiments performed in monkeys did not indicate that subsequent infection following vaccination were associated with enhanced viremia after the second vaccination.

In study DEN011Mk, different vaccine combinations were tested for their immunogenicity. Combination in which the first immunization was done with formulations containing "classical" live attenuated Vero Dengue Vaccine (VDV)-1 and VDV-2 viruses, followed by a secondary immunization with CYD viruses, were the ones which yielded the highest GMTs titres (3 to 100-fold higher that two vaccinations with the CYD viruses).

Secondary pharmacodynamic studies

Secondary pharmacodynamics studies were not performed as no specific risks were identified with CYD dengue vaccine, which is acceptable according to the EMA and WHO guidelines.

Safety pharmacology programme

Separate safety pharmacology studies were not conducted, which is acceptable according to the EMA and WHO guidelines. No specific risk was identified with CYD dengue vaccine, except the neutropic characteristics (neurovirulence) which were assessed in monkey studies (see Toxicology section in this report).

Pharmacodynamic drug interactions

No studies regarding drug interactions were performed in accordance with EMA and WHO guidelines.

2.3.3. Pharmacokinetics

Results of a distribution, persistence and shedding study in monkeys are discussed in the toxicology section. No other toxicokinetics studies were performed with CYD dengue vaccine, which is acceptable according to the EMA and WHO guidelines.

2.3.4. Toxicology

All toxicity studies were GLP compliant with the exception of the pilot investigative reproductive toxicity studies. Study phases from some toxicity studies (e.g. serology screening, immunogenicity) were conducted in non-GLP laboratories. The repeat-dose toxicity, biodistribution and shedding and neurovirulence studies were conducted in the non-human primate (NHP). The cynomolgus monkey was considered the relevant species since it is an established model for general toxicity assessment and it is the recommended species in the monkey safety tests for the evaluation of neurovirulence and viscerotropism of live attenuated yellow fever (YF) and dengue vaccines (WHO Technical Report Series (TRS) n° 872 and 979). The NHP also demonstrates a measurable immune response to CYD dengue vaccine.

Repeat dose toxicity

Nonclinical safety of CYD dengue vaccine after a single dose injection was evaluated as part of the repeat-dose toxicity study and the distribution and shedding study in the monkey. No systemic toxicity was observed in the repeat dose toxicity study, performed in one relevant animal species, cynomolgus monkeys, with comparable dose, route and frequency of administration as intended for human use (i.e. 3 subcutaneous administrations of 5 log10 CCID50 of each serotype in 0.5 mL). Local tolerance was assessed in the repeat-dose toxicity study and in the distribution and shedding study (see below). Occasional transient and minimal erythema reaction were noted at the injection site, which correlated with minimal to slight perivascular lymphocyte infiltration seen at the microscopic examination. These findings are expected and considered part of the intended immune response.

Genotoxicity and carcinogenicity

The absence of genotoxicity and carcinogenicity studies is considered acceptable based on the type of product and in line with current guidelines on non-clinical evaluation of vaccines.

Reproduction Toxicity

In the absence of a perfect animal model, investigative studies were performed to evaluate the suitability of the mouse and rabbit model for evaluation of reproductive and developmental toxicity. The rabbit was

selected to investigate the effects of the antibody response and exposure to repeated IV injections of CYD dengue vaccine at the human dose and the mouse was selected to investigate the effects of the exposure to the virus after one IV injection of CYD dengue vaccine at doses from 5 to 8 log10 CCID50 of each serotype.

These studies show that increasing the dose from the human dose to the maximal feasible dose of CYD dengue vaccine, and changing the route of administration from subcutaneous to IV injection, allow detection of viral RNA and antibody response to all serotypes in both species, and suggest the rabbit is most suitable for evaluation of antibody effects and the mouse for viremia. Dose range finding studies show antibody transfer (in the rabbit) and limited virus transfer (in the mouse) to developing offspring. The pivotal reproductive and developmental studies with IV injection of the human dose of the vaccine showed no adverse effects on the mating performance and fertility of the vaccinated rabbit, and no teratogenic potential and no effect on pre- and post-natal development in mouse and rabbit. The effects observed after IV injection of higher doses were observed only in association with maternal toxicity in the mouse. Considering the safety margin based on the absence of adverse findings after administration of a full human dose in rabbits and mice, no reproductive and developmental toxicity are expected.

Other toxicity studies

CYD dengue vaccine is a live attenuated vaccine and as such, its distribution, persistence and shedding were evaluated in cynomolgus monkeys after SC administration. Flavivirus-seronegative cynomolgus monkeys received a single SC administration of CYD dengue vaccine at approximately 5 log10 CCID50 of each serotype in 0.5 mL (phase III lot material), which corresponds to the human dose level and volume. The distribution data showed that CYD Dengue Vaccine RNA was predominantly limited to the injection site, the lymphoid tissues and liver, with detection in adrenals, bone marrow and skeletal muscle in occasional animals. There was evidence of viral clearance at day 21 after vaccination with persistence limited to very low level in injection site and draining lymph node samples in a few animals only. Absence of detection of viral RNA in the nervous system tissues supports lack of neurotropism. There was no shedding of CYD Dengue Vaccine RNA in body fluids. Dissemination to the environment or transmission from vaccinees to close contacts would therefore not be expected. Viremia, which is considered a marker of viscerotropism, was low and never exceed WHO acceptable limits for viremia.

All live dengue vaccines should be tested once for neurovirulence, which is a particular concern for dengue vaccine viruses derived from YF-17D. The neurotoxic profile of CYD dengue vaccine was evaluated over a 30-day period following single intracerebral administration to cynomolgus monkeys and compared to a yellow fever vaccine single intracranial injection, at a dose equivalent to the human dose, as requested in the WHO guidelines (WHO TRS n° 979). Clinical scores for encephalitis did not exceed the scores for yellow fever vaccine, and the histological scores were significantly lower. The assessment in monkeys correlated with mouse neurovirulence studies that were conducted as part of the manufacturing control of virus seed lots and the safety characterization of CYD dengue vaccine viruses, which also demonstrated that the recombinant vaccines were less neurovirulent for 8 day-old mice than YF 17D vaccine and not neurovirulent in young adult mice after injection by the intracerebral route. The neurotoxic profile of CYD dengue vaccine is therefore considered acceptable.

Vaccination with YF-17D vaccine is associated with the rare occurrence of acute viscerotropic disease. Although viral tropism is largely linked with the virus E protein, which is replaced by the dengue coding region for E protein in CYD viruses, viscerotropism was evaluated in this model of IC injected NHP by measure of viremia. The highest value in CYD dengue vaccinated monkeys was 3.3 log10 PFU/ml, thereby fulfilling the WHO criteria for absence of viscerotropism (WHO TRS n°872 and 979).

2.3.5. Ecotoxicity/environmental risk assessment

The environmental risk assessment was performed in accordance with Annex II to Directive 2001/18/EC on the deliberate release into environment of genetically modified organisms (GMOs) and following the precautionary principle using the methodology set down in Council Decision 2002/812/EC and Commission Decision 2002/623/EC and EMA guidelines on environmental risk assessments for medicinal products consisting of, or containing GMOs (EMEA/CHMP/BWP/473191/2006).

In accordance with Article 6 of Regulation (EC) No 726/2004, national competent authorities established under Directive 2001/18/EC have been consulted.

The risk assessment methodology of GMOs recognizes the following steps: (1) hazard identification, (2) hazard characterization, (3) assessment of likelihood, (4) risk estimation, (5) evaluation of risk management options followed by (6) a conclusion on the acceptability (or not) of the overall impact of the use of the GMO on human health and the environment taking into account the management strategies applied.

Beside direct effects of the GMO (e.g. pathogenicity or sensitization to subsequent wt DENV infection), indirect effects through which people who are not intended to be vaccinated and environment may become at risk were also described. These indirect effects may arise from a causal chain of events. Therefore, the potential hazard related to a) genetic instability and potential for reversion to virulence and b) recombination with wt flaviviruses due to homologous or non-homologous recombination and the formation of replication competent recombinants were considered important factors and as such included in the 'hazard identification step'.

The ERA performed is comprehensive and includes evaluations substantiated by data acquired during 15 years. *In vitro* and preclinical *in vivo* experiments in non-human primates have shown that there is limited risk of viscerotropism and neurotropism with CYD viruses compared with YF 17D, as expected. Reversion to virulence is an important aspect with live attenuated vaccines, in particular with RNA viruses. The CYD virus do not have YF 17D prM or E genes and carry numerous attenuating residues within the seven YF 17D non-structural genes and the capsid protein gene (in total 48 nucleotides sequence differences, 22 of which leading to amino acid substitutions). A recombination event or multiple mutational events that change the attenuated phenotype to one of virulence and simultaneously enhance the capacity of the virus to replicate, disseminate, and be transmitted by the mosquito are deemed to be highly unlikely. Furthermore, chimerisation compromises replication competence, underscoring the low probability that a vaccine/wt recombinant would possess a high mosquito infectivity phenotype.

Studies investigating the likelihood of intermolecular recombination between different flaviviruses *in vitro* indicate that recombination of the CYD vaccine viruses with a wt flavivirus is extremely unlikely. Furthermore, "worst-case scenarios" exchange mutants created ad-hoc (where whole vaccine construct's genes were swapped with wild type virus' genes) showed that replication and transmission in mosquitoes and outcomes in non-human primates were attenuated compared to wt viruses. Further reassurance is given by the fact that there is no evidence that the use of YF 17D in endemic regions has led to emergence of recombinant virus.

Should shedding occur (viral shedding data from two clinical studies CYD04 Phase I and CYD17 Phase III showed low and transient CYD dengue virus in urine and saliva in only a very low percentage of subjects), it will not contribute to the dissemination in human population as CYD Dengue viruses are fragile lipid-enveloped viruses sensitive to desiccation. They do not form survival structures nor replicate outside their human or mosquito host.

Taking into account the route of vector-borne transmission of flaviviruses, aspects such as the degree of viremia in a vaccinee and the ability of mosquitoes or ticks to transmit the CYD dengue viruses were evaluated to assess their dissemination in the environment. CYD dengue vaccine viremia was shown to be absent or present at low-levels and for a short duration in animal and human studies. Moreover, it has been shown that arthropods vectors such as mosquitoes or ticks were unable to transmit CYD dengue viruses after oral feeding.

Waste treatment and the minimum requirements for waste disposal were agreed during the procedure, as well as an emergency plan in case of accidental spill or exposure.

Considering all of these elements, there are no major objections linked to release of Dengvaxia into the environment.

2.3.6. Discussion on non-clinical aspects

The monkey was the species of choice for the pharmacology and toxicology studies. The limitation of this animal model is that monkeys do not develop symptomatic dengue disease upon infection. In view of the limited alternative options which also have their disadvantages, including immunocompromised mice presenting some symptomatic infection, this is considered acceptable. No major objections were identified and no additional studies are required.

Pharmacokinetic studies are normally not required for a vaccine. The Applicant provided a distribution, persistence and shedding study in monkeys but no other toxicokinetics studies were performed with CYD dengue vaccine, which is acceptable.

All pivotal toxicology studies have been conducted according to GLP requirements and the relevant EMA and WHO guidelines. Overall, the nonclinical safety data demonstrate that CYD dengue vaccine has an acceptable safety profile.

2.3.7. Conclusion on non-clinical aspects

Overall, the non-clinical safety data demonstrate that CYD dengue vaccine has an acceptable safety profile. The application is approvable from a non-clinical perspective.

2.4. Clinical aspects

2.4.1. Introduction

The present Application includes clinical data from 31 completed or ongoing Phase I to Phase III studies conducted in dengue endemic and non-endemic regions, representing data in more than 40,000 subjects from 9 months through 60 years of age exposed to at least one injection of the final tetravalent CYD dengue vaccine formulation. Immunogenicity data have been collected in the population from 9 months through 60 years. Pivotal efficacy data have been collected in children and adolescents from 2 to 16 years.

A tabular overview and listing of the main clinical studies are provided in Figure 4 and Table 3. In addition the following study was performed upon identification of a safety risk in sero-negative individuals: Phase IIIb/IV (Supplemental study) entitled Risk of symptomatic, hospitalized and/or severe VCD according to dengue serostatus in CYD Vaccine Efficacy Trials (CYD14, CYD15, CYD23/57). Refer to section 2.5.3 and relevant subsections.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the Applicant. A GCP inspection was conducted for CYD14 in 2016. Despite deficiencies in the monitoring process across all sites, which were addressed by the Applicant, the data collected were deemed of acceptable quality.

The Applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Figure 4: Overview of Clinical Development: Early Development, Pivotal and Supportive Studies

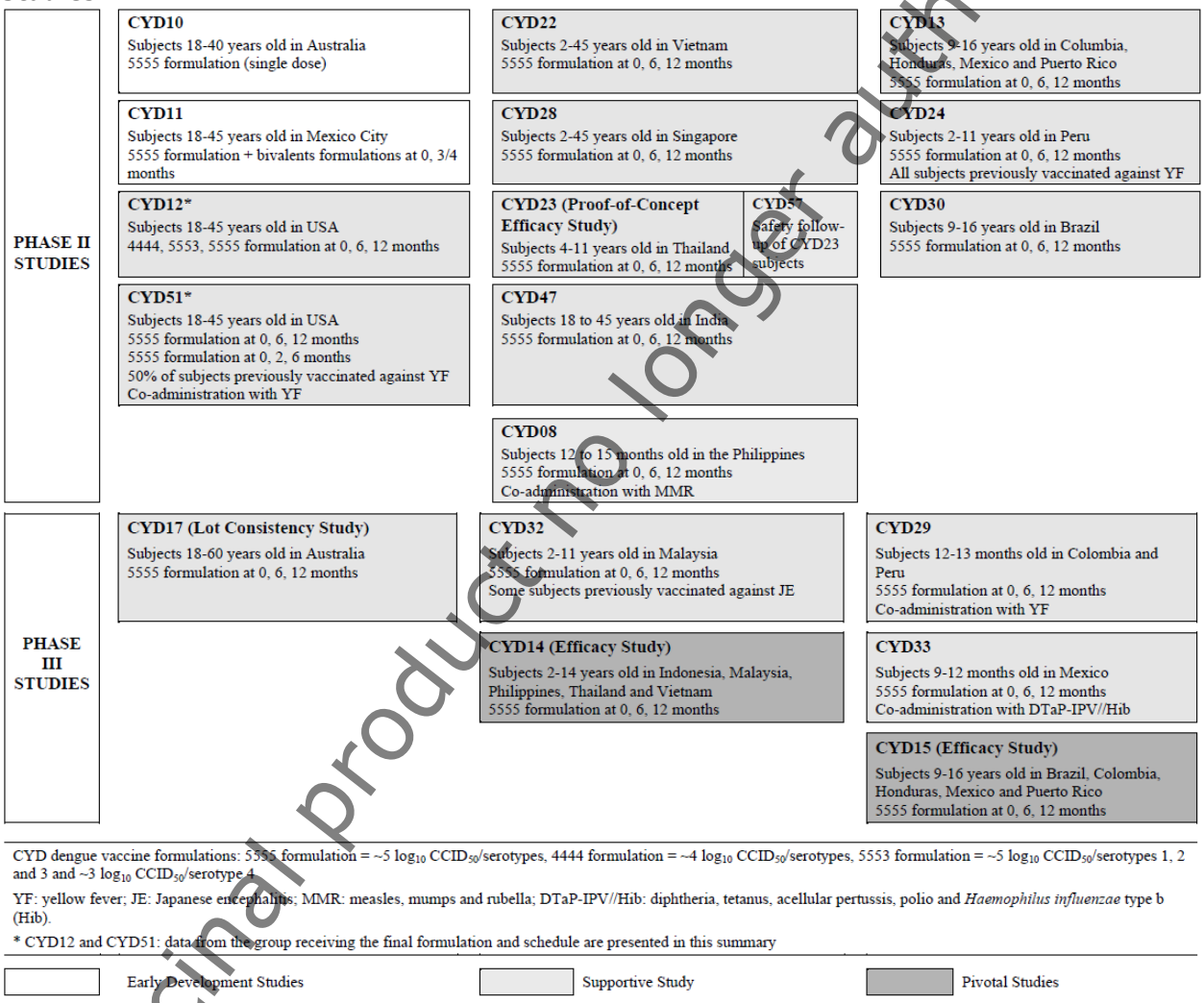


Table 3: Tabular Listing of all Clinical Studies

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
CYD01 Completed; Sponsor for this study: Acambis Inc	- Descriptive safety and tolerability. - Vaccine viremia. - Descriptive dengue humoral and cellular immune response. - **Antibody persistence** up to 1 year.	Phase I, monocenter, randomized, controlled, double-blind (open for yellow fever immune group) trial.	**ChimeriVax™-DEN2 vaccine** at D0 **Group 1: ~**5 log_{10}PFU/ serotype 2. **Group 2: ~**3 log_{10}PFU/ serotype 2. **Group 3: Yellow fever vaccine** (**YF-VAX®**) at D0. **Group 4** (subjects with previous YF vaccination)**: ~**5 log_{10}PFU/ serotype 2. 0.5 mL/injection. Subcutaneous injection.	Randomized: 42 yellow fever non-immune subjects + 14 yellow fever immune subjects enrolled without randomization in Group 4 - Group 1: 14 - Group 2: 14 - Group 3: 14 - Group 4: 14	USA Non-endemic area 05 Mar 2002 to 26 Jun 2002 (antibody persistence follow-up not included)	Healthy adults 18–49 years
CYD02 Completed; Sponsor for this study: Acambis Inc	- Descriptive safety and tolerability after each injection. - Vaccine viremia after each injection. - Descriptive dengue humoral immune response before and after each injection. - Effect of prior YF vaccination.	Phase I, monocenter, randomized, controlled, double-blind (1st injection), open (2nd injection) trial.	**CYD Dengue Vaccine (~4 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1**: CYD dengue vaccine at D0 and M5 to M9. **Group 2: Yellow fever vaccine** (YF-VAX®) at D0. CYD dengue vaccine at M5 to M9. **Group 3: Placebo** (YF-VAX® diluent) at D0. CYD dengue vaccine at M5 to M9. 0.5 mL/injection. Subcutaneous injection.	Randomized: 99 - Group 1: 33 - Group 2: 33 - Group 3: 33	USA Non-endemic area 17 Nov 2003 to 13 Nov 2004	Healthy adults 18–40 years
CYD04 completed	- Descriptive safety after each injection. - Vaccine viremia after each injection. -Viral shedding after a first injection. - Descriptive dengue humoral and cellular immune response before and after each injection.	Phase I, monocenter, randomized, placebo-controlled, blind-observer (1st injection), open (2nd & 3rd injections) trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M3.5 and M12 **Group 2: Placebo** (YF-VAX® diluent) at D0. CYD dengue vaccine at M3.5 and M12. **0.5 mL/injection.** **Subcutaneous injection.**	Randomized: 66 - Group 1: 33 - Group 2: 33	USA Non-endemic area 11 Oct 2005 to 13 Feb 2007	Healthy adults 18–45 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
CYD05 Completed; Interim CSR up to 28 days after the 3rd injection + CSR Addendum for antibody persistence data up to 5 years after the 3rd injection	- Descriptive safety after each injection. - Vaccine viremia after each injection. - Descriptive dengue humoral immune response before and after each injection. - 5-year post-injection 3 follow-up: antibody persistence and safety. - Detection of symptomatic dengue cases during the first 4 years of follow-up.	Phase I, monocenter, randomized, controlled, blind-observer (1st injection), open (2nd & 3rd injections) trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M3.5 and M12. **Group 2: Typhoid vaccine** (Typhim Vi®) at D0. CYD dengue vaccine at M3.5 and M12. **0.5 mL/injection.** **Subcutaneous injection.**	Randomized: 126 Group 1: 84 • 12 adults • 24 adolescents (12–17 years) • 24 children (6–11 years) • 24 children (2–5 years) Group 2: 42 • 6 adults • 12 adolescents (12–17 years) • 12 children (6–11 years) • 12 children (2–5 years)	Philippines Endemic area 02 Mar 2006 to 11 Sept 2012 (including 5 years follow-up after the 3rd injection)	Healthy subjects 2–45 years
CYD06 completed	- Descriptive safety after each injection. - Vaccine viremia after each injection. - Descriptive dengue humoral immune response before and after each injection.	Phase I, multicenter, randomized, controlled, blind-observer (1st injection), open (2nd & 3rd injections) trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M3.5 and M12. **Group 2: Yellow fever vaccine** (Stamaril Pasteur®) at D0. CYD dengue vaccine at M3.5 and M12. **0.5 mL/injection.** **Subcutaneous injection.**	Randomized: 126 Group 1: 84 • 12 adults • 24 adolescents (12–17 years) • 24 children (6–11 years) • 24 children (2–5 years) Group 2: 42 • 6 adults • 12 adolescents (12–17 years) • 12 children (6–11 years) • 12 children (2–5 years)	Mexico Non endemic area 24 Jan 2006 to 20 Aug 2007	Healthy subjects 2–45 years
CYD10 completed	- Descriptive safety after one injection. - Vaccine viremia after one injection. - Descriptive dengue humoral and cellular immune response before and after one injection.	Phase IIa, monocenter, controlled, open trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** All groups: **CYD dengue vaccine at D0.** **Group 1**: subjects who received monovalent Vero dengue vaccine, VDV1 (serotype 1) or VDV2 (serotype 2) 1 year before inclusion (in a previous study).	Enrolled subjects: 35 - Group 1: 15 - Group 2: 8 - Group 3: 12	Australia Non-endemic area 02 Aug 2006 to 13 Mar 2007	Healthy subjects 18–40 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
	- 6 months post-injection safety follow-up.		**Group 2**: subjects who received yellow fever vaccine 1 year before inclusion (in a previous study). **Group 3**: flavivirus non-immune subjects. **0.5 mL/injection.** Subcutaneous injection.			
CYD11 Completed Final CSR + Addendum to CSR with PRNT data (retest)	- Descriptive safety after each injection. - Vaccine viremia after each injection. - Descriptive dengue humoral and cellular immune response before and after each injection. - 12 months post-injection 1 safety follow-up.	Phase IIa, multicenter randomized, controlled, open trial.	**Bivalent or tetravalent CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype)** **Blending tetravalent CYD dengue vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 3, 4) + Vero dengue vaccine (~4 $log_{10}CCID_{50}$/serotype 2**) **Group 1**: Bivalent CYD vaccine (1, 3) at D0. Bivalent CYD vaccine (2, 4) at M3.5. **Group 2:** Bivalent CYD vaccine (1, 3) + bivalent CYD (2, 4) at D0 and M3.5. **Group 3:** Blending tetravalent vaccine at D0 and M3.5. **Group 4:** Tetravalent CYD dengue vaccine at D0 and M3.5. **Group 5: JE vaccine[2]** (JE-VAX®) at D-14, D-7 and D0. Tetravalent CYD dengue vaccine at M3.5. - Bivalent and tetravalent CYD, and blending tetravalent CYD/VDV: 0.5 mL/injection. - JE vaccine: 1.0 mL/injection. Subcutaneous injection.	Randomized: 155 - Group 1: 30 - Group 2: 31 - Group 3: 30 - Group 4: 32 - Group 5: 32	Mexico Non-endemic area 11 Aug 2008 to 30 Oct 2009	Healthy subjects 18–45 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
CYD12 completed	- Descriptive dengue humoral immune response before and after each injection. - Descriptive safety, after each injection. - Vaccine viremia after the first and second injections. - 6 months post-injection 3 safety follow-up.	Phase II, randomized, double-blind, multicenter trial.	**CYD Dengue Vaccine formulations:** **5555** (~5 $log_{10}CCID_{50}$/serotype 1, 2, 3, 4) **5553** (~5 $log_{10}CCID_{50}$/serotype 1, 2, 3) and (~3 log_{10} $CCID_{50}$/serotype 4) **4444** (~4 log_{10} $CCID_{50}$/serotype 1, 2, 3, 4) **Group 1:** CYD dengue vaccine (5555) at D0, M6 and M12. **Group 2:** CYD dengue vaccine (5553) at D0, M6 and M12. **Group 3:** CYD dengue vaccine (4444) at D0, M6 and M12. 0.5 mL/injection. Subcutaneous injection.	Randomized: 260 - Group 1: 104 - Group 2: 103 - Group 3: 53	USA Non-endemic area 17 Apr 2008 to 14 Dec 2009	Healthy subjects 18–45 years
CYD13 completed	- Descriptive dengue humoral immune response before and after each injection. - Descriptive safety after each injection. - Detection of symptomatic dengue cases. - 6 months post-injection 3 safety follow-up.	Phase II, randomized, controlled, blind-observer (1st and 2nd injections), single blind (3rd injection), multicenter, multinational trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0 and M6. **Tdap[3] vaccine** (ADACEL®) at M12. 0.5 mL/injection Placebo and CYD dengue vaccine: subcutaneous injection. Tdap vaccine: intramuscular injection.	Randomized: 600 - Group 1: 401 - Group 2: 199	Colombia Honduras Mexico Puerto Rico Endemic areas 09 Oct 2009 to 29 Aug 2011	Healthy subjects 9–16 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[i])	Healthy Subjects or Diagnosis of Patients
CYD22 Completed; Final CSR (up to 4 years post injection 3)	- Descriptive dengue humoral immune response before and after each injection. - Descriptive safety, after each injection. - 4-year post-injection 3 follow-up: antibody persistence and safety. - Detection of symptomatic dengue cases.	Phase II, randomized, controlled, blind-observer, monocenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$ / serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Meningococcal Polysaccharide A+C vaccine** at D0. **Placebo** (NaCl 0.4% containing human serum albumin 2.5%) at M6. **Typhoid Vi Polysaccharide vaccine** (Typhim Vi®) at M12. 0.5 mL/injection. Subcutaneous injection.	Randomized: 180 Group 1: 120 • 20 adults • 20 adolescents • 40 children (6–11 years) • 40 children (2–5 years) Group 2: 60 • 10 adults • 10 adolescents • 20 children (6–11 years) • 20 children (2–5 years)	Vietnam Endemic area 14 Mar 2009 to 12 Jul 2014 (including 4 years post-injection 3 follow-up)	Healthy subjects 2–45 years
CYD24 Completed; Final CSR + Addendum to CSR with PRNT Data (retest)	- Descriptive dengue humoral immune response, before and after each injection, in children previously vaccinated against yellow fever. - Descriptive safety after each injection. - Vaccine viremia, after the first and second injections, in a subset of subjects. - Detection of symptomatic dengue cases. - 6-month post-injection 3 safety follow-up.	Phase II, randomized, controlled, blind-observer, monocenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.4% containing human serum albumin 2.5%) at D0 and M6. **Pneumococcal polysaccharide vaccine** (Pneumo23®) at M12. 0.5 mL/injection. Subcutaneous injection.	Randomized: 300 (but 2 not vaccinated) Group 1: 199 • 99 children (6–11 years) • 100 children (2–5 years) Group 2: 99 • 49 children (6–11 years) • 50 children (2–5 years)	Peru Endemic area 26 Sep 2008 to 16 Aug 2010	Healthy subjects 2–11 years
CYD28 completed	- Descriptive safety after each injection. - Descriptive dengue humoral response before and after each injection in a subset of subjects. - Descriptive cellular immune response after the 2nd and 3rd injection in a subset of subjects.	Phase II, randomized, controlled, blind-observer (1st injection), single blind (2nd and 3rd injection), multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2:** *If < 12 years* **Placebo** (NaCl 0.9%) at D0. **Hepatitis A vaccine** (Havrix®) at M6 and M12. *If ≥ 12 years*	Randomized: 1198 Group 1: 898 • 521 adults • 141 adolescents • 236 children Group 2: 300 • 174 adults	Singapore Endemic area 07 Apr 2009 to 14 Oct 2014; (including 4 years post-injection 3	Healthy subjects 2–45 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[i])	Healthy Subjects or Diagnosis of Patients
	- 4-year post-injection 3 follow-up: antibody persistence (in a subset of subjects) and safety. - Detection of symptomatic hospitalized dengue cases.		Placebo (NaCl 0.9%) at D0. **Influenza vaccine** (Vaxigrip®) at M6 and M12. 0.5 mL/injection. Subcutaneous injection for all but Hepatitis A vaccine: intramuscular injection.	• 46 adolescents • 80 children	follow-up)	
CYD30 completed	- Descriptive dengue humoral immune response before and after each injection. - Descriptive safety after each injection. - Detection of symptomatic dengue cases. - 6-month post-injection 3 safety follow-up.	Phase II, randomized, placebo-controlled, blind-observer, monocenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/injection. Subcutaneous injection.	Randomized: 150 Group 1: 100 • 60 adolescents (12 to 16 years) • 40 children (9 to 11 years) Group 2: 50 • 31 adolescents (12 to 16 years) • 19 children (9 to 11 years)	Brazil Endemic area 20 Aug 2010 to 15 May 2012	Healthy subjects 9–16 years
CYD47 completed	- Descriptive dengue humoral immune response before the 1st injection and after each injection. - Descriptive safety after each injection. - Detection of symptomatic dengue cases. - 6-month post-injection 3 safety follow-up.	Phase II, randomized, placebo-controlled, blind-observer, multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/injection. Subcutaneous injection.	Randomized: 189 - Group 1: 128 - Group 2: 61	India Endemic area 27 Mar 2012 to 07 Dec 2013	Healthy subjects 18–45 years
CYD23 completed	- Vaccine efficacy against virologically confirmed dengue cases. - Descriptive dengue humoral immune response, before and after each injection and one year after the 3rd injection, in a subset of subjects.	Phase IIb, randomized, controlled, blind-observer, monocenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine - cohort 1: at D0, M6 and M12. - cohort 2: at D0, M6 and M12. **Group 2:** - cohort 1**: Rabies vaccine** (Verorab®) at D0. **Placebo** (NaCl 0.9%) at M6 and M12.	Randomized: 4002 Two-step enrollment as per cohort number : Group 1: 2669 • 100 in cohort 1 • 2569 in cohort 2	Thailand Endemic area 05 Feb 2009 to 22 Mar 2012 (13 months after injection 3: end	Healthy subjects 4–11 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
	- Safety throughout the trial and descriptive reactogenicity (injection site and systemic), after each injection, in a subset of subjects. - Vaccine viremia, after the 1st and 2nd injections, in a subset of subjects.		- cohort 2: Placebo at D0, M6 and M12. 0.5 mL/ injection. Subcutaneous injection.	Group 2:1333 • 50 in cohort 1 • 1283 in cohort 2	of Active Phase) End of the study (after a hold): 10 Sep 2013	
CYD57 Ongoing; Interim CSR up to 2 years post injection 3 received in CYD23	- 4-year post-injection 3 safety follow-up of subjects previously enrolled in CYD23. - Detection and characterization of hospitalized dengue cases. - Evaluation of occurrences of related (linked to CYD dengue vaccine received in CYD23) and fatal SAEs.	Monocenter, safety follow-up study of CYD23.	No vaccine administration.	Included: 3203 Group 1: 2131 Group 2: 1072 (subjects included in CYD23)	Thailand Endemic area 10 Sep 2013 (after hold of CYD23) to 17 Feb 2014 (24 months post-injection 3 follow-up) Planned completion date including 5-year post-injection 3 follow-up: Mar 2016	Healthy subjects 4–11 years at enrollment in CYD23
CYD17 Completed; Final CSR + Addendum to CSR with exploratory analysis	- Lot-to-lot consistency across 3 Phase III lots. - Bridging between Phase II and Phase III lots. - Descriptive safety, after each injection. - Vaccine viremia, after each injection, in a subset of subjects. - Virus shedding, after each injection, in a subset of subjects. - Descriptive dengue humoral immune response	Phase III, randomized, placebo-controlled, blind-observer, multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Groups 1, 2 and 3**: CYD dengue vaccine Phase III lots 1, 2, 3 respectively at D0, M6 and M12. **Group 4**: CYD dengue vaccine Phase II lot at D0, M6 and M12. **Group 5: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/ injection. Subcutaneous injection.	Randomized: 715 - Group 1: 164 - Group 2: 163 - Group 3: 163 - Group 4: 168 - Group 5: 57	Australia Non-endemic area 05 Oct 2010 to 12 Jun 2012	Healthy subjects 18–60 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[i])	Healthy Subjects or Diagnosis of Patients
	after the 3rd injection, according to the flavivirus immune status at baseline in a subset of subjects. - 6-month post injection 3 safety follow-up.					
CYD32 completed	- Descriptive safety, after each injection. - Descriptive dengue humoral immune response, after the 2nd and 3rd injection. - 6-month post-injection 3 safety follow-up.	Phase III, randomized, placebo-controlled, blind-observer, multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotypes 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/ injection. Subcutaneous injection.	Randomized: 250 Group 1: 199 • 99 (2–5 years) • 100 (6–11 years) Group 2: 51 • 26 (2–5 years) • 25 (6–11 years)	Malaysia Endemic area 02 Dec 2010 to 14 Aug 2012	Healthy subjects 2–11 years
CYD14 completed; Interim CSR up to 48 months post-injection 3 (Year 3 Hospital Phase) submitted	- Vaccine efficacy against virologically confirmed dengue cases. - Safety throughout the trial and descriptive reactogenicity (injection site and systemic) after each injection, in a subset of subjects. - Descriptive dengue humoral immune response, after the 2nd and 3rd injection, in a subset of subjects. - 5-year post-injection 3 follow-up: safety, detection of confirmed hospitalized dengue cases and antibody persistence in a subset of subjects.	Phase III, randomized, placebo-controlled, blind-observer, multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/ injection. Subcutaneous injection.	Randomized: 10,275 - Group 1: 6851 - Group 2: 3424	Indonesia, Malaysia, Thailand, the Philippines, Viet Nam Endemic areas 03 Jun 2011 to 05 Dec 2014 (24- month post-injection 3 follow-up) Planned completion date including 5-year post-injection 3 follow-up: Nov 2017	Healthy subjects 2–14 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
CYD15 Ongoing; Interim CSR up to 48 months post-injection 3 (Year 3 Hospital Phase) submitted	- Vaccine efficacy against virologically confirmed dengue cases. - Safety throughout the trial and descriptive reactogenicity (injection site and systemic) after each injection, in a subset of subjects. - Descriptive dengue humoral immune response, after the 2nd and 3rd injection, in a subset of subjects. - 5-year post-injection 3 follow-up: safety, detection of confirmed hospitalized dengue cases and antibody persistence in a subset of subjects.	Phase III, randomized, placebo-controlled, blind-observer, multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12. **Group 2: Placebo** (NaCl 0.9%) at D0, M6 and M12. 0.5 mL/ dose. Subcutaneous injection.	Randomized: 20,869 - Group 1: 13,920 - Group 2: 6949	Brazil, Colombia, Honduras, Mexico, Puerto Rico Endemic area 08 Jun 2011 to 04 Mar 2015 (24-month post-injection 3 follow-up) Planned completion date including 5-year post-injection 3 follow-up: Apr 2018	Healthy subjects 9–16 years

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
CYD08 completed	- Descriptive safety, after each injection (dengue and/or MMR[4] vaccines). - Vaccine viremia after the first dengue injection. - Descriptive humoral immune response (dengue and/or MMR vaccines) after each respective injection. - Detection of symptomatic dengue cases. - 6-month post-injection 3 safety follow-up.	Phase II, randomized, controlled, modified double-blind (1st injection), open (2nd and 3rd injections), multicenter trial.	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** MMR (Trimovax®) at M-1. CYD dengue vaccine at D0, M6 and M12. **Group 2:** MMR at M-1. Varicella vaccine (Okavax®) at D0. Hepatitis A vaccine (Avaxim®) at M6 and M12. **Group 3:** Varicella vaccine at M-1. CYD dengue vaccine **+** MMR at D0. CYD dengue vaccine at M6 and M12. **Group 4:** MMR at M-1. CYD Dengue vaccine + Placebo (NaCl 0.9%) at D0. CYD Dengue vaccine at M6 and M12. All groups: DTaP-IPV/Hib vaccine[5] (Pentaxim®) at M9. 0.5 mL/ injection. CYD dengue vaccine, MMR, Varicella and Placebo: subcutaneous injection. Hepatitis A and DTaP-IPV/Hib vaccines: intramuscular injection.	Randomized: 210 - Group 1: 60 - Group 2: 30 - Group 3: 60 - Group 4: 60 Three-step enrollment as per cohort number: ▪ Cohort 1: - Group 1: 60 - Group 2: 30 ▪ Cohort 2: - Group 3: 20 - Group 4: 20 ▪ Cohort 3: - Group 3: 40 - Group 4: 40	Philippines Endemic area 18 Jan 2010 to 08 May 2012	Healthy subjects 12–15 months at first injection
CYD29 completed	- Non-inferiority of the immune response against yellow fever (YF) in subjects receiving one injection of YF vaccine concomitantly with 1st injection of CYD dengue vaccine compared to one injection of YF with placebo. - Descriptive safety, both after the injection of the YF vaccine (concomitantly with placebo or CYD dengue vaccine) and after	Phase III, randomized, blind-observer multicenter trial [not controlled for dengue vaccine but placebo-controlled per design for the evaluation of the concomitant vaccine].	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine + Yellow Fever vaccine (Stamaril®) at D0. CYD dengue vaccine at M6 and M12. **Group 2:** Placebo (NaCl 0.9%) and Yellow Fever vaccine at D0. CYD dengue vaccine at M6 and M12. All subjects: - MMR (Trimovax®) + PCV[6] (Prevenar13®) + Hepatitis A (Avaxim®) at M1. - DTaP-IPV/Hib (Pentaxim®) at M7. - Hepatitis A at M13.	Randomized: 792 - Group 1: 396 - Group 2: 396	Peru, Colombia Endemic areas 07 Sep 2011 to 02 Sep 2013	Healthy subjects 12–13 months at first injection

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
	each CYD dengue vaccine injection, for all subjects. - Descriptive YF humoral immune response for all subjects. - Descriptive dengue humoral immune response in a subset of subjects. - 6-month post-injection 3 safety follow-up.		0.5 mL/ injection Placebo, YF, MMR and CYD dengue vaccine: subcutaneous injection. PCV, Hepatitis A and DTaP-IPV/Hib vaccine: intramuscular injection.			
CYD33 completed	- Non-inferiority of the immune response against all antigens (diphtheria, tetanus, pertussis, polio and Hib) in subjects receiving a booster dose of Pentaxim concomitantly with the 2nd injection of CYD dengue vaccine compared to one booster dose of Pentaxim concomitantly with placebo. - Descriptive safety after the injection of the Pentaxim booster dose (concomitantly with placebo or with the 2nd injection of CYD dengue vaccine) and after each CYD dengue vaccine injection, for all subjects. - Descriptive dengue humoral immune response to each dengue serotype after the 2nd and 3rd injection in a subset of subjects. - Vaccine viremia after the first dengue injection. - 6-month post-injection 3	Phase III, randomized, open-label (1st and 3rd injection), blind-observer (2nd injection), multicenter trial [not controlled for dengue vaccine but placebo-controlled per design for the evaluation of the concomitant vaccine].	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0. CYD dengue vaccine + DTaP-IPV/ Hib vaccine (Pentaxim®) at M6. Placebo at M7. CYD dengue vaccine at M12. **Group 2**: CYD dengue vaccine at D0. DTaP-IPV/Hib + Placebo (NaCl 0.9%) at M6. CYD dengue vaccine at M7 and M12. All subjects: MMR + PCV at M1. 0.5 mL/ injection. Placebo, MMR and CYD dengue vaccine: subcutaneous injection. PCV and DTaP-IPV/Hib vaccine: intramuscular injection.	Enrolled : 720 Randomized: 624 - Group 1: 309 - Group 2: 315	Mexico Endemic area 18 Jul 2011 to 04 Feb 2014	Healthy subjects 9-12 months at first injection

Study Identifier and study status/ report	Main Objectives of the Study	Study design	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Countries; Endemic / Non-endemic Area; Trial Period (FVFS – LVLS[1])	Healthy Subjects or Diagnosis of Patients
	safety follow-up.					
CYD51 completed	- Descriptive dengue humoral immune response to each dengue serotype after the 3rd injection of two different vaccine schedules in naïve or previous YF vaccinated subjects. - Descriptive antibody persistence, in naïve or already YF vaccinated subjects, 6-month post-injection 3. -Descriptive YF humoral immune response at baseline and 28 days after each injection of CYD dengue vaccine in YF+ subjects in Groups 1 and 2 -Descriptive YF humoral immune at baseline and 1, 3, and 7 months after injection of the YF vaccine at D0 in Groups 3 and 4 -Descriptive safety profile after each injection of CYD dengue vaccine and/or YF vaccine.	Phase II, randomized, open-label, multicenter trial	**CYD Dengue Vaccine (~5 $log_{10}CCID_{50}$/ serotype 1, 2, 3, 4)** **Group 1:** CYD dengue vaccine at D0, M6 and M12 **Group 2:** CYD dengue vaccine at D0, M2 and M6 **Group 3:** CYD dengue vaccine at D0, M2 and M6 **+** YF[7] at D0. **Group 4**: YF at D0.	Randomized: 390 - Group 1: 120 - Group 2: 120 - Group 3: 120 - Group 4: 30 For groups 1 and 2: - 60 subjects without previous YF vaccination - 60 with previous YF vaccination.	USA Non-endemic area 06 Dec 2011 to 27 Sep 2013	Healthy subjects 18–45 years

[1] FVFS-LVLS: first visit of the first subject– last visit of the last subject (LVLS includes last contact of subjects by telephone call)
[2] JE: Japanese encephalitis
[3] Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Absorbed
[4] MMR: Measles, Mumps, Rubella vaccine
[5] DTaP-IPV/Hib: Diphtheria, tetanus, Pertussis, Poliomyelitis and Hib vaccine
[6] PCV: pneumococcal conjugate vaccine
[7] YF: yellow fever

2.4.2. Pharmacokinetics

For vaccines, biopharmaceutics concerns the bioavailability of the vaccine components after administration. In accordance with the CHMP "Guideline on Clinical Evaluation of New Vaccines", pharmacokinetic studies (including bioavailability and bioequivalence studies) are usually not required for vaccines.

The main characteristics of the *in vivo* behaviour of the live CYD dengue vaccine have been evaluated through assessment of vaccine viremia and shedding. These are also important endpoints for the evaluation of the safety profile of the vaccine. Vaccine viremia is defined as the presence of vaccine viruses in the blood stream. Viremia and virus shedding were assessed using the same high sensitivity approach, i.e. screening with non-serotype specific RT PCR followed by serotype specific identification with CYD RT PCRs and/or plaque assay (PA) depending on the study.

Vaccine viremia

Post-vaccination vaccine viremia was investigated in nonclinical and some clinical studies as an assessment of safety, but also as a measure of the bioavailability and replicative ability of the vaccine virus. Nonclinical studies showed that the predominant CYD serotype inducing measurable and reproducible viremia upon tetravalent CYD immunization was CYD-4, with measurable viremia due to other serotypes rarely observed. This viremia was low following the first injection, and no viremia was observed after the second dose. CYD-1 or CYD-3 vaccine strains occasionally induced viremia, but only as a monovalent or bivalent vaccine. Further details are provided in 2.6.2 Pharmacology Written Summary.

During the clinical development of the CYD dengue vaccine, vaccine viremia was assessed at several timepoints after each injection in some studies, generally in a subset of subjects, with Plaque Assay (CYD04, CYD05, CYD06, CYD10 and CYD11) and with quantitative RT-PCR (CYD04, CYD05, CYD06, CYD08, CYD10, CYD11, CYD12, CYD24, CYD23, CYD17 and CYD33), in order to determine the timing, peak and duration of viremia. These methods are widely used within the field, since they are able to detect and quantify vaccine viremia and they are in accordance with WHO recommendations for monitoring of vaccine viremia. As Plaque Assay was not a validated assay, only results obtained with quantitative RT-PCR were considered.

An integrated analysis in subjects who received one or several injections of CYD dengue vaccine was performed to summarize quantified viremia, as the assays for the assessment of viremia were validated at the level of quantitation. After a first injection of CYD dengue vaccine, few subjects (3.8%) across these pooled studies had non serotype-specific vaccine viremia as assessed by RT-PCR. The proportion of subjects with measurable non serotype-specific viremia decreased with subsequent injections. After the second injection, vaccine viremia was less frequently observed than after the first injection, and almost no viremia was identified after the third injection.

Vaccine viremia appeared to have short duration after each CYD dengue vaccine injection; it generally occurred around D7 and never after D14. CYD-4 was the most frequently identified vaccine virus serotype after a first injection of CYD dengue vaccine across studies.

Vaccine viremia incidence was low whatever the dengue immune status at baseline and whatever the age group. No safety concerns were associated with vaccine viremia.

Viral shedding

The spread of the CYD viruses in the environment after vaccination depends on the occurrence of vaccine virus shedding. To assess a possible shedding of CYD dengue vaccine virus in humans, urine and saliva were selected since YF vaccine virus secretion in urine has been reported and wild-type dengue virus has been detected in urine and saliva after infection. Virus shedding was explored in a small number of subjects in one Phase I study (CYD04) and as a more systematic evaluation in a larger subset (95 subjects receiving the CYD dengue vaccine) in the Phase III study CYD17. Flavivirus non-immune subjects were chosen as the presence of antibodies against flaviviruses can reduce the levels of vaccine viremia and virus shedding. The methods of detection of flavivirus RNA by RT-PCR and NS1 antigen in urine and saliva are very well documented and are acceptable.

Overall, the occurrence of vaccine virus shedding was rare, at low level and transient. The results in the Phase I study CYD04 indicated no vaccine virus shedding in the subset of 11 CYD dengue vaccine and 5 control subjects that was assessed after the first injection. Data on virus shedding assessed in urine and saliva by RT-PCR available from CYD17 showed that vaccine virus shedding was observed in urine samples from 2 subjects at levels close to the lower limits of quantification (LLOQs). No replication-competent viruses were identified in these samples. No safety concerns were associated with viral RNA shedding.

2.4.3. Pharmacodynamics

As for any vaccine in accordance with current vaccines guidelines, the pharmacodynamic profile for the CYD dengue vaccine was defined by its immunogenicity profile in healthy subjects.

The principal targets for Ab response to wild-type dengue virus infection in human are the prM and E structural proteins and the non-structural NS1 protein. Ab response in both primary and secondary dengue virus infections is predominantly directed against E protein and in humans is highly cross-reactive across serotypes and exhibit neutralizing activity. Ab to the prM protein can bind partial or immature particles; they are highly cross-reactive and exhibit a weak neutralizing capacity. NS1-specific Ab are highly cross-reactive. Cell-mediated immune responses as well as complement-dependent lysis of infected cells are induced after infection by wild type dengue virus. The immunogenicity profile of the CYD dengue vaccine was therefore assessed through the measurement of humoral and cellular immune response.

Mechanism of action

Dengvaxia contains live attenuated viruses. Following administration, the viruses replicate locally and elicit neutralizing antibodies and cell-mediated immune responses against the four dengue virus serotypes.

Criteria for assessment of humoral responses

No immune correlate of protection is currently established for dengue. The measurement of immune responses to flaviviruses/flavivirus vaccines has classically been assessed by determining the level of neutralizing Ab, which has been correlated with protection against flavivirus diseases including YF and Japanese encephalitis (JE). Accordingly, functional neutralizing Ab titres were measured to assess dengue vaccine immunogenicity. In addition anti-NS1 IgG and total IgM/IgG were also measured.

PRNT assay

The plaque reduction neutralizing test (PRNT) is considered the most specific method for dengue vaccine immunogenicity testing in line with WHO recommendation. The PRNT method applied by the Applicant during early development was subsequently optimized and revalidated between Phase I and Phase II to

align with current industry standards for assay validation; the optimized PRNT50 method was the core immunologic assay for measuring functional antibodies able to inactivate and neutralize dengue virus since it was utilized throughout Phase II and onwards through clinical development. The assay methodology is in line with WHO recommendation, which is acceptable. Matched vaccine antigens have been used within the assay. Inter-site assay variability has been avoided by centralizing the serology assessment in the Applicant's laboratory (for all studies that assessed the final formulation) and implementing appropriate internal standards. The PRNT assay was also used to determine the presence of YF and JE antibodies in vaccinees.

The principle is that functional antibodies are able to neutralise dengue virus. The assay measures the amount of functional antibodies in human sera based on the number of foci induced by an infectious dengue challenge virus on cells. The reported value (end point neutralization titre) represents the highest dilution of serum at which ≥50% of dengue challenge virus (in foci counts) is neutralized when compared to the mean viral foci count in virus control wells which represents the 100% virus load.

A dengue PRNT with a higher stringency (PRNT90) was used during evaluation to reanalyse blood samples for post-hoc efficacy analyses by dengue immune status at baseline. Using a more stringent assay may likely lead to lower false positive rate resulting from Flaviviruses cross-reactivity, but it would also run the risk of a higher false negative rate. Serological cross-reactivity amongst members of the Flaviviridae family (Dengue (DENV), Yellow Fever (YF), West-Nile virus (WNV), Japanese Encephalitis virus (JEV) and Tick borne encephalitis virus (TBEV)) is a well-known diagnostic problem. A PRNT50 titre may provide more accurate results from the linear portion of the titration curve, but it is inevitably more variable. For these reasons, serological conversion (either using PRNT or ELISA) was not used as an endpoint to determine if a subject was infected with Dengue, rather an algorithm of virological confirmation using Dengue RT-PCR and NS1 antigen ELISA were utilized in the phase III studies as the specificity and sensitivity were superior.

The following parameters were used to characterize the humoral immune response induced by the CYD dengue vaccine:

- Geometric mean of titres (GMTs) expressed in reciprocal of dilution (1/dil) for each serotype
- Geometric mean of titre ratio (GMTRs) from baseline to post-vaccination for each serotype
- Seropositivity rate, defined as the proportion of subjects with a neutralizing Ab titre ≥10 (1/dil). This level also corresponds to the lower limit of quantification (LLOQ) of the PRNT assay. Seropositivity rate was assessed for each serotype and cumulatively for at least one, two, three and four serotypes.

Based on experience with the CYD dengue vaccine in dengue endemic populations of different ages and regions, GMT became the most important criteria for the dose assessment and for the assessment of the effect of covariates on antibody response. As an analysis of covariates, levels of neutralizing Ab were also assessed:

- by baseline JE/YF immune status defined as follows:
  - Immune: subjects with quantified (≥ 10 [1/dil], the LLOQ) neutralizing antibodies against JE or YF in the baseline sample.
  - Non-immune: subjects without quantified (< 10 [1/dil]) neutralizing antibodies against JE or YF, depending on the region, in the baseline sample. For non-endemic regions, JE or YF were not considered so baseline dengue/JE/YF status is also baseline dengue status.
- by baseline dengue immune status defined as follows:

- Immune: subjects with quantified (≥ lower limit of quantitation [LLOQ]) neutralizing Ab against at least one dengue serotype in the baseline sample.
- Non-immune: subjects without quantified (< LLOQ) neutralizing Ab against any of the four dengue serotypes in the baseline sample.

The baseline dengue immune status became the covariate of interest in terms of immunogenicity status at baseline.

IgM/IgG ELISA assay

This assay was used to assess the serological profile of suspected dengue cases (see endpoints). However it was considered as supportive and not used as a primary endpoint assay to determine if a subject had been infected with Dengue. This is due to the demonstrated cross-reactivity of antibodies directed against both the Dengue envelope and NS1 proteins with antibodies to other flaviviruses which circulated in the region or are part of routine vaccination programs, such as JEV, West Nile, Zika and Yellow Fever. As described by recent articles, serological diagnosis has good sensitivity (97.1%), but low specificity (85.1%) compared to virological confirmation.

The IgG ELISA was performed using a commercially available kit. The principle of this kit is based upon exposing sera to dengue Ags that are attached to the surface of the ELISA plate. Dengue specific IgG Abs bound to the dengue Ags are detected by the addition of an anti-IgG MAb complexed to HRP, which following addition of a substrate effects a colorimetric change that is detected by the ELISA reader. The IgM ELISA and IgG ELISA tests were to be applied to all samples from all dengue suspected cases from D0 until Vse, regardless of time of event after vaccination, whereas for samples from Vse until the end of the trial testing by IgM/IgG ELISA tests was not mandatory. An anti-NS1 ELISA was also used to differentiate between subjects infected by wt dengue and subjects vaccinated in a post-hoc supplemental study (see section 2.5.3).

Assessment of cell mediated immune responses

The role of CMI in clearing natural flavivirus infection is well established, but its implication in vaccination and subsequent protection against pathogen challenge in dengue is poorly understood.

In order to further characterize the immune response induced by the CYD dengue vaccine and as recommended in WHO and CHMP guidelines, cell-mediated immunity was assessed in some studies in adolescents and adults in endemic and non-endemic regions (studies CYD04, CYD10, CYD11 and CYD28). Specific cytokines in supernatants of purified peripheral blood mononuclear cells (PBMCs) stimulated *in vitro* with live vaccine of each serotype were measured essentially by cytometric bead array (CBA) and by measuring the frequency of antigen-specific CD4 and CD8 cells by intracellular cytokine staining (ICS). There was no evidence of increase in inflammatory responses after immunization with the CYD dengue vaccine. Dominance of the cellular response to serotype 4 was observed after first dose of the vaccine, however the response was balanced against all four serotypes following 3 injections. These serotype-specific T cell responses paralleled the neutralizing Ab responses measured by PRNT50 assay. Regarding the cytokine profile, the vaccine induced a cellular response with a Th1/Tc1 profile wherein interferon-γ (IFN-γ) dominates over tumor necrosis factor α (TNF-α) and Th2 cytokines including interleukin-13 (IL-13).

2.4.4. Conclusion on clinical pharmacology

Adequate studies were performed to determine vaccine viremia and measuring of the immunogenicity of the vaccine. These studies followed WHO recommendations. Vaccine viremia and vaccine shedding were

found to be minimal, and therefore no specific precautions need to be taken with the vaccines and their contacts.

No immune correlate of protection is currently established for dengue but based on current knowledge it was considered adequate that immunogenicity assessment for CYD vaccine was based on neutralising antibody titres. Neutralizing antibodies are known to be important for protection against JE and YFV. The use of a validated plaque reduction neutralization test (PRNT) to determine the immunogenicity of the vaccine was considered adequate. PRNT90 is more specific than PRNT50 with regard to cross-reacting antibodies against flaviviruses. The use of GMTs, GMTRs and seropositivity rates as the parameters to characterize the immune response induced by the vaccine is acceptable.

2.5. Clinical efficacy

The CDP followed the WHO guidelines available at the time of initiating the clinical trials as well as scientific advice provided by EMA, FDA and several national regulatory authorities. Immunogenicity of the vaccine was studied in 16 trials, three of which were carried out to determine clinical efficacy due to lack of immunological correlate of protection for dengue: a proof of concept phase IIb study CYD23/57 and two large pivotal phase III studies CYD14 and CYD15 that were initiated in 2011. All 3 studies were conducted in dengue endemic areas (Latin-America and Asia-Pacific) and in total they recruited approximately 35,000 subjects aged 2 to 16 years.

During the application several submissions were received and assessed based on different analyses at different cut off points. Therefore, some of the data presented in this section are a mix of final and intermediate results.

2.5.1. Dose response studies

A total of 5 Phase I studies (CYD01, CYD02, CYD04, CYD05, CYD06) and 3 Phase II studies (CYD10, CYD11 and CYD12), conducted at the beginning of the clinical development, investigated different vaccine potency, doses and time interval of administration leading to the selection of the final vaccine formulation (~5 log10 CCID50 of each serotype) and the final vaccination schedule (3 injections given 6-month apart). The CYD dengue vaccine was initially developed in subjects from 2 years of age, thus the choice of the formulation, schedule and dosing interval was done to ensure that all subjects, dengue immune or dengue non-immune at baseline, had an immune response to all 4 dengue serotypes.

- CYD01 assessed safety and immunogenicity of a single dose of monovalent chimeric dengue 2 vaccine containing 5 or 3 $\log_{10}$ plaque forming units [PFU], and showed that satisfactory immune responses could be achieved against serotype 2 but low seropositivity rates to the other 3 serotypes (in YF non-immune subjects), confirming the need of a tetravalent vaccine.
- CYD02: tested a tetravalent formulation with 4 log10 CCID50 per serotype (2 doses given at 5 to 9 month interval) induced moderate but unbalanced Ab levels against the four serotypes.
- CYD04: tested a tetravalent formulation with 5 log10 CCID50 per serotype (3 doses), showing satisfactory safety and immunogenicity profiles in FV non-immune adults. See below.
- CYD05 and CYD06: tested a tetravalent formulation as above in different age groups (2 to 45 years) and FV backgrounds. Immunogenicity responses was achieved against all four serotypes but varied due to age, baseline status, region, ranging from 39.1% (CYD04, FV non-immune adults) to 85.0% (CYD05, FV immune adults, adolescents, and children).

- CYD11 tested the use of sequential or simultaneous bivalent formulations, which did not improve the immune response compared to the tetravalent formulation.
- In CYD12, the immunogenicity of 3 vaccine formulations was assessed: 5555 (5 log10 for each of the 4 serotypes), 5553 (5 log10 for serotypes 1, 2, and 3 and 3 log10 for serotype 4), and 4444 (4 log10 for each of the 4 serotypes). The 5553 formulation was intended to improve the immune response by taking into account the immunodominance of serotype 4 observed in previous studies. The 5555 formulation showed a trend toward higher seropositivity rates to the 4 serotypes after the third injection (62.9%), compared to the other formulations. The different vaccine formulations showed that different concentrations of a given serotype can impact the immune response to the other serotypes.
- CYD10 was an immunogenicity and safety study in 18-45YOA with a single dose of the 5555 formulation. CMI was assessed in this study, in addition to studies CYD04, CYD11 and CYD28.

In non-endemic populations, an immune response based on anti-dengue Ab GMTs and seropositivity rates [Ab titer ≥ 10 1/dilution (dil)] against all 4 dengue serotypes was observed only after 3 doses of CYD dengue vaccine (CYD04). A stepwise increase in seropositivity rates against each serotype was observed at each dose of the 3-dose schedule at 0, 3-4, and 12 months. A more robust immune response was observed in children, and a potential priming effect was observed following administration of YF vaccine (CYD06). Overall subjects from non-endemic areas (and therefore assumed to be mainly dengue seronegative at baseline) who received three doses of vaccine responded poorly in terms of GMT titres and percentage of subjects who seroconverted to all 4 dengue serotypes. These subjects reached lower GMTs than those from endemic areas, which was also observed in other Phase II and III trials.

In endemic populations, an immune response against all 4 dengue serotypes was also observed after 3 doses of CYD dengue vaccine (CYD05). A similar stepwise increase in seropositivity rates against each serotype with higher GMTs in people previously exposed to wild type dengue was observed. Moreover, two doses administered over a longer interval (at 0 and 8–9 months) in people previously exposed to WT dengue induced a similar immune response as that of the 3-dose schedule.

The final formulation (5555 with ~5 log10 CCID50 per serotype) induced the highest levels of GMTs and also the highest rates of seropositivity rates (62.9% of subjects were seropositive to the 4 serotypes after the third injection). Based on these results, further clinical development for the endemic indication was based on the 5 log10 CCID50 per serotype, with the exception of 2 studies that evaluated bivalent/tetravalent blending of dengue vaccines (CYD11) and differing concentrations per serotype with a 0, 6, and 12-month schedule (CYD12) (i.e. contributing to dose-ranging). Further, the 3-dose schedule (0, 3-4, and 12 months) and 2-dose schedule (0 and 8–9 months) were adapted to adjust for the higher immunogenicity that occurred when Dose 2 was delayed, balanced by providing protection as soon as possible.

Thus the 5 log10 CCID50 dosage and the 3-dose schedule (0, 6, and 12 months) were selected for subsequent Phase II and Phase III trials for the endemic country indication (except Study CYD11).

Late Phase II Studies

Based on safety and immunogenicity results from the above-mentioned studies, 5 additional Phase II studies (CYD13, CYD22, CYD24, CYD28, CYD30) were performed in different endemic countries in AP and LatAm to further evaluate the safety and immunogenicity of the CYD dengue vaccine in different populations (i.e. by age, baseline JE or YF vaccination status, region) following 3 injections of the final formulation administered 6 months apart. A proof-of-concept efficacy study (Phase IIb) was then conducted in Thailand (CYD23) in children aged 4 to 11 years, for whom a safety follow-up was done

(CYD57). An additional Phase II study was performed in India (CYD47) to assess safety and immunogenicity of the CYD dengue vaccine in Indian subjects 18-45YOA, as required by local Authorities for registration. Some of these studies are further discussed in the following sections.

A proof-of-concept co-administration Phase II study (CYD08) was also conducted to evaluate the co administration of CYD dengue vaccine together with measles/mumps/rubella (MMR) vaccine in toddlers below 2 years of age.

2.5.2. Main studies

There is currently no vaccine authorised in Europe to protect against dengue and no correlate of protection (CoP) yet identified. Therefore the development of a tetravalent dengue vaccine required generating efficacy data in a dengue endemic population at risk of infection by these viruses.

The clinical development of the CYD dengue vaccine contains 3 studies evaluating the efficacy of the vaccine to protect against symptomatic dengue disease. As recommended in the CHMP and WHO Guidelines, a proof of concept supportive Phase IIb efficacy study (CYD23) in Thailand was performed, followed by 2 large-scale pivotal Phase III efficacy studies (CYD14 and CYD15) in different regions (AP and LatAm, respectively) and in numerous countries. As per the WHO guideline each one of the 3 efficacy studies was statistically powered for the primary efficacy endpoint, i.e. prevention of occurrence of symptomatic virologically confirmed dengue (VCD) cases due to any serotypes 28 days after the third injection, regardless of severity. The Phase IIb study was designed to provide a proof of efficacy in one centre from one particular country, while the aim of the Phase III program was to provide confirmatory efficacy data in various dengue epidemiology settings in Asia and LatAm. Each Phase III efficacy study was therefore designed to generate data in a particular region in terms of endemicity and age group. All 3 studies were conducted using the final formulation of the CYD dengue vaccine administered 6 months apart. Efficacy was also assessed after each injection and per serotype, and against severe VCD cases, either according to WHO grading or according to the assessment of the Independent Data Monitoring Committee (IDMC), and against hospitalized VCD cases.

This section summarizes the methodology and efficacy data obtained in preventing the occurrence of VCD cases in individual studies CYD23, CYD14 and CYD15. Integrated and meta-analysis of combined data from CYD14 and CYD15 were evaluated to support the results of individual studies and/or to provide more precision for some endpoints on subcategories of VCD cases or some covariates, as well as sensitivity analysis on all efficacy data.

The choice of the study countries and sites for the phase III trials was based on national surveillance data and available data from epidemiological studies showing that these countries were highly endemic ad have had evidence of al 4 serotypes circulating. The choice was confirmed by the results of 2 prospective, active fever surveillance, cohort studies conducted by the Applicant prior to the initiation of the studies in Latin America (Brazil, Colombia, Honduras, Puerto Rico and Mexico) and Asia (Indonesia, Malaysia, Philippines, Thailand and Vietnam). These data provided an estimate of the dengue attack rate in the study target population (3.4% of VCD cases in AP and 1.2% of VCD in LatAm) and trained the sites in conducting active surveillance of symptomatic dengue cases in school settings or through direct contact with subjects and families.

Study titles and design

CYD23: Efficacy and Safety of Dengue Vaccine in Healthy Children Aged 4 to 11 Years in Thailand.

Study design CYD23

CYD23 was a randomized, blind-observer, controlled Phase IIb study conducted in 1 centre in Thailand. A two-step approach to enrolment in 2 cohorts was performed for safety purposes. In total, 4002 healthy children (4 to 11 years) were randomized into 2 groups: 2668 subjects were to receive 3 injections, 6 months apart, of the CYD dengue vaccine and 1334 subjects were to receive either one injection of a rabies vaccine (Verorab) followed by 2 injections of a placebo at 6 and 12 months (50 children) or 3 injections, 6 months apart, of a placebo (1284 children). Long-term follow-up of safety and hospitalized dengue cases was evaluated through the extension CYD57 Study.

CYD14: Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 14 years in Asia.

Study Design CYD14

This was a Phase III efficacy trial with a randomized, observer-blind, placebo-controlled, multicentre design in 11 sites in 5 different countries in Asia. A total of 10,278 children aged 2 to 14 years were enrolled into the trial to receive 3 injections at 0, 6, and 12 months and to be randomized in a 2 to 1 ratio so that 6,852 subjects would receive CYD dengue vaccine and 3,426 would receive a placebo.

- Subsets CYD14

A subset of subjects from each country were evaluated for reactogenicity and immunogenicity to enable the generation of country-specific data on reactogenicity, immunogenicity, and baseline dengue and Japanese encephalitis (JE) antibody (Ab) levels. Subjects were randomized to the subset during the first 2 months of enrolment in each country. Between 300 and 600 subjects were targeted to be enrolled in each participating country, to a total of 2,000 subjects (1,333 in the CYD Dengue Vaccine Group and 667 in the Control Group).

CYD15: Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children and Adolescents Aged 9 to 16 years in Latin America.

Study Design CYD15

This is a Phase III efficacy trial with a randomized, observer-blind, placebo-controlled, multicentre design in 22 sites across 5 countries in Latin America. Children and adolescents aged 9 to 16 years received 3 vaccinations at 0, 6, and 12 months and were randomized in a 2 to 1 ratio so that 13,917 subjects were to receive CYD dengue vaccine and 6,958 were to receive a placebo.

- Subsets CYD15

A subset of subjects from each country was evaluated for reactogenicity and immunogenicity to enable the generation of country-specific data on reactogenicity, immunogenicity, and baseline dengue and YF Ab levels. The immunogenicity and reactogenicity subset included a total of 2,000 subjects (1,334 in the CYD Dengue Vaccine Group and 666 in the Control Group).

Methods

With the aim of evaluating the protective effect of CYD dengue vaccine (~5 $\log_{10}$ $CCID_{50}$ of each serotype), one group of subjects received three doses of the CYD dengue vaccine and the other group

received 3 doses of placebo. Placebo injections consisted of NaCl 0.9%. Both vaccine and placebo were injected via the subcutaneous route. A vaccination schedule of 0, 6, and 12 months was chosen based on Phase I and Phase II trial results, to optimize the immune response of the dengue vaccine after the second and third injections. The period from the first injection (V01) to 28 days after the third injection (V06) was defined as the vaccination period.

The observer-blind design was chosen since the products had different aspects and could be recognized. The person who performed vaccinations knew which product had been administered while neither the subject nor the Investigator in charge of safety evaluation, nor the Sponsor, nor the parents/guardians of subjects did know which product had been injected. The "vaccinator" was in charge of preparing and administering the products and had to ensure that the documents on randomization were stored in a secure place where only he/she had access.

The design of the control group was based on the need to maintain the blind to minimize any potential bias in the evaluation of the primary objective of the study (i.e., efficacy evaluation). The placebo had to use the same route (subcutaneous [SC]) and the same schedule as the study vaccine (0, 6, and 12 months) otherwise the study could be de facto unblinded.

Case ascertainment in CYD14/CYD15 studies was performed in 2 phases (see Figure 5):

1. The "**Active Phase**" lasted from injection 1 until 13 months following the third injection, and two endpoint were studied: i) symptomatic virologically confirmed dengue cases regardless of severity, occurring more than 28 days after completion of the vaccination schedule (primary objective), and ii) severe dengue and hospitalized dengue. It was expected that the number of symptomatic VCD cases in a 12-month period was sufficient to demonstrate efficacy. As this period began after 28 days after Dose 3, the Active Phase of dengue surveillance continued for each subject until 13 months after Dose 3. Active surveillance was utilised during this phase and it was designed to maximize the detection of all potential symptomatic dengue cases. It included weekly contacts with subjects or subject's parent(s) by phone calls/SMS or home visits, and school absenteeism surveillance. The purpose of this contact was to provide a reminder to parents to take the child to the trial centre or health care centre in the event of febrile illness. Passive (spontaneous consultation) detection of febrile episodes was also implemented. Two blood samples (acute and convalescent) were to be taken to confirm the dengue case in the event of any acute febrile illness during the Active Phase. The first blood sample (acute) was to be taken throughout the trial during the acute phase of the disease, as soon as possible (within 5 days in CYD14/CYD15 and within 7 days in CYD23) after the onset of fever. The second blood sample (convalescent) was to be taken between 7 and 14 days after the acute sample. Acute samples were used for the virological confirmation of dengue cases (see primary endpoint).

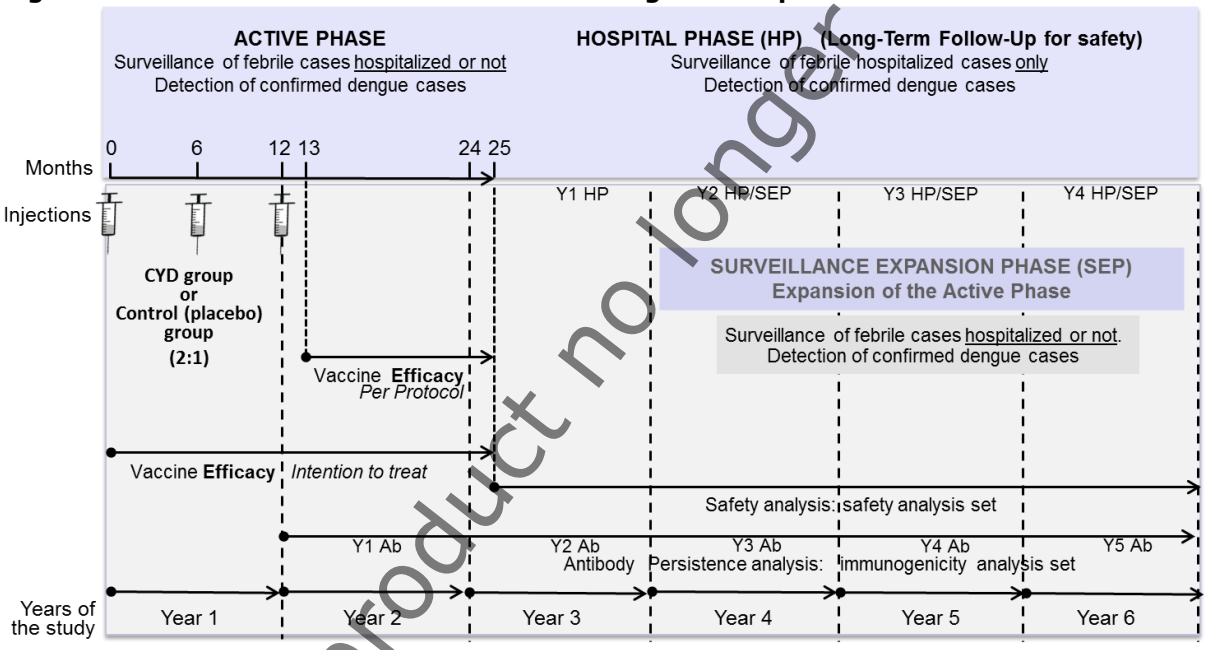
   This phase lasted from 08 June 2011 to 03 April 2014 (ca. 25 months per subject).

2. The "**Hospital Phase**" was a long term follow-up period of semi-active/passive surveillance for 4 years after the end of the Active Phase (i.e. 5 years after the last injection) to collect **hospitalized dengue** cases (severe and non-severe). In January 2015, CYD14 and CYD15 protocols were amended to maximize the detection of all symptomatic dengue cases during the long-term follow-up and to provide additional information on long-term efficacy of the CYD dengue vaccine. The process of reconsenting the subjects to an active surveillance system (surveillance expansion phase [SEP]) was started in at least one site by May 2015 in CYD15 and by June 2015 in CYD14. The SEP was fully implemented in all trials of CYD14 and CYD15 in June 2016 with almost 80% of participants in CYD15 and about 90% of participants in CYD14 accepting participation in the SEP. During the Hospital Phase, participants were to attend yearly visits, and

establish at least ≥1 contact every 3 months, by phone, short messages services (SMS) or home visit. Subjects could also present to and/or contact the Investigators at any time. Hospitalization for acute fever was recorded during study contacts and visits, and through self-reporting and surveillance of identified non-study hospitals. Acute and convalescent samples were to be taken in the event of hospitalized acute febrile illness during the Hospital Phase until the Surveillance Expansion visit, when the subject was to consent or decline to enter the SEP. Subjects that declined to participate were to continue surveillance and acute/convalescent sampling until trial completion (up to 60 months post-injection 3). Subjects that consented to take part in the SEP were to be actively followed for dengue case detection similarly as during the Active Phase. As such, active surveillance is mimicking that of the Active Phase. However, blood sampling triggered by identified febrile illnesses during the SEP is encompassing only collection of acute samples. This phase lasted from 15 March 2012 to 04 March 2015 (ca. 47 months per subject).

3. **Extension phase** (2015 – 2018): the surveillance expansion phase was completed towards the end of the evaluation procedure, thus the final data will be assessed post-approval.

Figure 5: Outline of CYD14 and CYD15 trial design and important timelines



Data presented in this section cover the Active Phase and the first year of the Hospital Phase (i.e., from 13 months to 25 months after the third injection).

CYD23 was initially planned as an efficacy and safety study performed in 2 phases:

- The "Active Phase" lasted from the start of the trial to 13 months after the third injection, and two endpoints were studied: i) Symptomatic VCD cases, and ii) severe dengue. 05 February 2009 (first visit fist subject) - 22 March 2012 (end of Active phase)
- The "Passive Phase" was intended as a long term immunogenicity and safety follow-up period designed for a period of 3 years after the end of the Active Phase. However, following a request from a Public Health Authority, the CYD23 study was stopped at the beginning of the Passive Phase. All subjects included in CYD23 study were asked to take part in a separate long-term follow-up study (CYD57 study), which investigated hospitalized dengue and safety follow-up up to 4 years after the end of the Active Phase (i.e. including a retrospective data collection from the end of the Active Phase of CYD23 until the CYD57 study start and a prospective data collection from CYD57 study start until the end of the CYD57 study).

Blood sampling for immunogenicity

In study CYD23, baseline antibody titres were evaluated in blood samples obtained from 300 study subjects (non-randomly selected).

In CYD14 and CYD15, only a random subset of subjects provided a pre-vaccination sample (20% and 10% of subjects, respectively). These subjects were designated as the "immunogenicity subset". Subjects in the 3 studies were to receive vaccine or placebo injections at enrolment, M6, and M12. All subjects were to provide a blood sample approximately 28 days after the third injection, although this sample was tested only in a subset of participants (those in the immunogenicity subset and those subjects developing VCD during follow-up). The purpose of this sample was to have a post-vaccination specimen in subjects who later developed confirmed dengue as part of the assessment of the relationship between neutralizing Ab levels and VE. Therefore, it was tested against each of the four parental dengue virus strains of CYD dengue vaccine among subjects who later developed virologically-confirmed dengue infection.

Study Participants

Trial CYD23 included subjects aged 4 years to 11 years of age. Trial CYD14 enrolled healthy children 2-14YOA from endemic regions of Thailand, Malaysia, Indonesia, Philippines, and Vietnam. Healthy children and adolescents aged 9 to 16 years living in endemic Dengue regions of Latin America were enrolled in CYD15 (Brazil (5 sites), Colombia (9 sites), Honduras (1 site), Mexico (5 sites), and Puerto Rico (2 sites)). The age ranges selected for enrolment corresponded to the ages with the highest incidence of clinical dengue reflecting the epidemiological situation by country or region at the time of the study conduct.

The criteria for inclusion and exclusion in the trials are described below:

- Diagnosis and main criteria for inclusion (CYD14 study and CYD15):
    1. Aged 2 to 14 years (CYD14 study), and Aged 9 to 16 years (CYD15 study), and aged 4 to 11 years (CYD23) on the day of inclusion and resident of the site zone;
    2. Subject in good health, based on medical history and physical examination;
    3. Assent form or informed consent form has been signed and dated by the subject (based on local regulations), and informed consent form has been signed and dated by the parent(s) or another legally acceptable representative (and by an independent witness if required by local regulations);
    4. Subject able to attend all scheduled visits and to comply with all trial procedures.
- Subjects who met any of the following main exclusion criteria were not included (CYD14 and CYD15 studies, and with minor modifications also applied to study CYD23):
    1. Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination until at least 4 weeks after the last vaccination);
    2. Participation in another clinical trial investigating a vaccine, drug, medical device, or a medical procedure in the 4 weeks preceding the first trial vaccination;
    3. Planned participation in another clinical trial during the present trial period;

4. Self-reported or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months; or long-term systemic corticosteroids therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months);
5. Self-reported seropositivity for Human Immunodeficiency Virus (HIV) infection;
6. Self-reported systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances;
7. Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion;
8. Receipt of blood or blood-derived products in the past 3 months, which might interfere with assessment of the immune response;
9. Planned receipt of any vaccine in the 4 weeks following any trial vaccination.

In phase I and Phase II studies, personal or family history of thimic pathology (thymoma), thymectomy, or myasthenia were exclusion criteria due a risk factor described for viscerotropism (for YF vaccines). Since no safety concerns were shown, these exclusion criteria were not included in Phase III studies and are not therefore considered to be a contraindication for CYD dengue vaccine.

All subjects were asked whether they had a history of YF vaccination or infection or dengue infection. However, baseline serostatus was only determined in the immunogenicity and reactogenicity subset.

Objectives

Primary Objective

To assess the efficacy of the CYD dengue vaccine after 3 injections administered 6 months apart in preventing the occurrence of symptomatic virologically-confirmed dengue (VCD) cases, regardless of the severity, due to any of the 4 serotypes, in children 2-14YOA in study CYD14 and in children 9-16YOA in study CYD15.

The assessment period extended from 28 days after the third injection to the end of the Active Phase (i.e. dengue surveillance continued for each subject until 13 months post-dose 3 (PD3 period)).

Secondary Objectives

ALL SUBJECTS (N=10,278 for CYD14 and N=20,875 for CYD15):

Efficacy during the Active Phase:

1. To describe the efficacy of CYD dengue vaccine in preventing symptomatic VCD cases after the third dose to the end of the Active Phase: a. due to at least 3 serotypes; b. due to each of the 4 serotypes.
2. To describe the efficacy of CYD dengue vaccine in preventing symptomatic VCD cases after at least 1 dose: a. due to any of the 4 serotypes, b. due to at least 3 serotypes, c. due to each of the 4 serotypes.
3. To describe the efficacy of CYD dengue vaccine in preventing symptomatic VCD cases after 2 doses: a. due to any of the 4 serotypes, b. due to at least 3 serotypes, c. due to each of the 4 serotypes.

Safety:

4. To describe the occurrence of SAEs, including serious adverse events of special interest (AESIs), in all subjects throughout the trial period.
5. To describe the occurrence of hospitalized virologically-confirmed dengue cases and the occurrence of severe (clinically-severe or as per WHO criteria) VCD cases, throughout the Surveillance Expansion period (SEP) and throughout the trial (from D0 until the end of the trial).

IMMUNOGENICITY AND REACTOGENICITY SUBSET (N=2,000 for each study):

Immunogenicity

6. To describe the Ab response to each dengue serotype after Dose 2, after Dose 3, and 1, 2, 3, 4 and 5 years after Dose 3.

Reactogenicity

7. To describe the reactogenicity of CYD dengue vaccine after each dose.

Other Objectives

Detection of dengue cases during the Active Phase

1. To describe the efficacy of CYD dengue vaccine in preventing VCD that meets WHO criteria for DHF, due to any of the 4 serotypes after each dose.
2. To describe the efficacy of CYD dengue vaccine in preventing clinically-severe VCD cases due to any of the 4 serotypes after each dose.
3. To describe the incidence of hospitalized VCD cases due to each or any of the 4 serotypes.
4. To describe the efficacy of CYD dengue vaccine in preventing symptomatic VCD cases due to each or any of the 4 serotypes between each dose.

Detection of dengue cases during the Hospital phase/Surveillance Expansion period

5. To describe the efficacy of CYD dengue vaccine in preventing symptomatic VCD cases, hospitalized cases and severe cases, due to each or any of the 4 serotypes after at least 1 dose.
6. To assess the risk factors associated with hospitalization and severity of VCD cases.

Serological profile of suspected dengue cases

7. To describe the serological profile of suspected dengue cases from D0 until the Surveillance Expansion period visit (Vse).

Dengue viremia

8. To describe the wild-type (WT) dengue strain viremia level in acute samples of VCD cases.

Relationship between neutralizing Ab level and vaccine efficacy

9. To describe the relationship between post-Dose 3 neutralizing Ab level and the subsequent occurrence of symptomatic dengue cases.
10. To describe the relationship between neutralizing Ab level at the time of SEP and the subsequent occurrence of symptomatic dengue cases of any severity, hospitalized, or severe VCD cases, during the SEP.

Medical and non-medical resource utilization related to dengue disease

11. To describe the level of medical and non-medical resource utilization linked to hospitalized and ambulatory confirmed dengue cases (first 25 months).

Relationship between Dengue and Zika (only for study CYD15)

12. To determine the occurrence of virologically confirmed Zika cases in febrile cases reported from start of 2013 (retrospectively) until the end of the trial, as a differential diagnosis for dengue infection.
13. To describe the clinical manifestations of Zika disease according to treatment group (CYD Dengue Vaccine or Control group), by Zika virus clade and overall.
14. To describe the antibody responses to dengue and Zika in blood samples taken in defined periods targeting prior to the first serologically-confirmed Zika cases reported by national surveillance systems and after the peak incidence of observed Zika cases or at the end of the trial if the epidemic is still on-going.

Exploratory analyses performed: Time-to-Event Analysis, Subgroup Analyses by age, country, baseline status, other covariates and covariates adjustment, description of clinical signs and symptoms of VCD cases.

Overall the objectives are in line with the WHO recommendation for the clinical development of dengue vaccines. Serious dengue disease was a secondary safety endpoint in the overall population of the pivotal trials, but in the immunogenicity subset clinical analysis was only exploratory due to the limited sample size.

CYD23 objectives

The primary objective was to assess the efficacy of the CYD dengue vaccine after 3 injections in preventing the occurrence of symptomatic VCD cases. Part of the secondary objectives was to evaluate the safety (reactogenicity in a subset and SAEs in all subjects) and the humoral immune response to the CYD dengue vaccine after each injection in a subset of subjects. Persistence of Ab levels was also evaluated up to 1 year after the third injection in the immunogenicity subset. Long-term follow-up of safety and hospitalized dengue cases was evaluated through CYD57 Study.

Endpoints

Primary endpoint

The primary endpoint in CYD14, CYD15 and CYD23 was symptomatic VCD cases occurring > 28 days after Dose 3 (during the Active Phase) and defined as an acute febrile illness (i.e. temperature ≥ 38°C on at least 2 consecutive days) virologically-confirmed by dengue RT-PCR and/or dengue NS1 ELISA Ag test.

Characterisation of suspected cases was carried out by testing of the acute blood sample, and included dengue non-structural protein (NS) 1 antigen (Ag) ELISA, dengue screen (DS) RT-PCR, dengue serotype specific RT-PCR (Simplexa), haematocrit, platelet count, aspartate aminotransferase (AST) and alanine transaminase (ALT). Dengue immunoglobulin (Ig) M/IgG ELISA was also performed in acute samples. Testing of the convalescent blood sample included dengue IgM/IgG ELISA, haematocrit, platelet count, AST and ALT.

The endpoints used in each individual study were used in the integrated efficacy analysis (IEA).

Secondary endpoints

1. Incidence of symptomatic VCD cases occurring > 28 days after Dose 3 to the end of the Active Phase due to at least 3 serotypes and due to each of the 4 serotypes
2. Incidence of symptomatic VCD cases after at least 1 dose due to any of the 4 serotypes, due to at least 3 serotypes, due to each of the 4 serotypes (i.e. efficacy 28 days after Dose 1 included dengue cases from > 28 days after Dose 1 until the end of the Active Phase)
3. Incidence of symptomatic VCD cases after 2 doses due to any of the 4 serotypes, due to at least 3 serotypes, due to each of the 4 serotypes
4. Reactogenicity and safety endpoints of subjects (refer to section 2.6)
5. Blood samples had to be collected from a subset of subjects (immunogenicity subset) and from all subjects at M13. For subjects in the immunogenicity subset (humoral immunity), the following assays were planned at specified time points: Neutralizing Ab level against each of the 4 parental dengue virus strains of CYD dengue vaccine constructs (and potentially against recently isolated strains) at baseline, after Dose 2, after Dose 3, and 1, 2, 3, 4 and 5 years after Dose 3 (dengue neutralization assay). Likewise, neutralizing Ab level was also measured on samples obtained at the time of consenting to the SEP. In addition, baseline neutralizing Abs against JE or YF was described depending on the study.

Other endpoints

1. Dengue cases during the Active Phase were taken into account if they occurred more than 28 days after respective vaccination. For example, efficacy 28 days after Dose 1 included dengue cases from > 28 days after Dose 1 until the end of the Active Phase.
2. The Surveillance Expansion Period Endpoint consisted of symptomatic VCD, hospitalized VCD cases and severe (clinically severe or as per WHO criteria) VCD cases observed during the SEP.
3. Serological Profile of Suspected Dengue Cases Endpoint: the serological profile of suspected dengue cases was based on IgG and IgM ELISA results.
4. Viremia Endpoint: WT dengue strain viremia level was measured in acute samples by quantitative DS RT-PCR.
5. Neutralizing Ab level against each of the 4 parental dengue virus strains of CYD dengue vaccine constructs were measured after the third injection and after the Vse at least, in the immunogenicity and reactogenicity subset, and in the subjects with a confirmed dengue infection. These neutralizing Ab titres were used to explore a potential predictive threshold or any correlate of risk associated with the observed vaccine efficacy in the trial.

As for the primary endpoint, the other endpoints used in each individual study were used in the IEA.

Case definitions

For the definition of symptomatic VCD refer to the primary endpoint.

The definition of dengue haemorrhagic fever (DHF) grade I, II, III, and IV was consistent with the 1997 WHO definition.

For ascertaining severe dengue cases according to the IDMC criteria, the Investigator had to consider the following potential manifestations of severity in all virologically-confirmed dengue cases and all dengue cases were reviewed by the IDMC who ensured consistent application of the term severe. The IDMC

Definition of Severe Dengue Fever (December 15, 2010) is: proven Dengue Fever (two days fever + virological confirmation) plus one of the following criteria:

- Platelet count ≤100 000 µl and bleeding (tourniquet, petechiae or any bleeding) plus plasma leakage (effusion on chest x-ray or clinically apparent ascites including imaging procedures or haematocrit ≥20% above baseline recovery level or standard for age if only one reading)
- Shock (pulse pressure ≤20 mmHg in a child, or hypotension [≤90 mmHg] with tachycardia, weak pulse and poor perfusion)
- Bleeding requiring blood transfusion
- Encephalopathy i.e. unconsciousness or poor conscious state or convulsions not attributable to simple febrile convulsion, as defined in the guidelines for definition and collection of febrile convulsions, or focal neurological signs. Poor conscious state or unconsciousness must be supported by Glasgow Coma Scale (GCS) score
- Liver impairment (AST >1000 U/L or prothrombin time [PT] International normalized ratio [INR] >1.5)
- Impaired kidney function (Serum creatinine ≥1.5 mg/dL)
- Myocarditis, pericarditis or heart failure (clinical heart failure) supported by chest X-ray (CXR), echocardiography, electrocardiogram (ECG) or cardiac enzymes where these are available.

For criteria 2 to 7 it was essential to check for co-morbidities. The presence of these did not exclude the case from being classified as severe. This definition applied to the cases reviewed by the IDMC. Cases with obvious other causes for the criteria were reviewed on a case by case basis.

Ascertainment of VCD cases

Surveillance methods aimed at detecting acute febrile illness (i.e. temperature ≥38°C on at least 2 consecutive days) considered as suspected dengue episode during the Active Phase or hospitalized acute febrile illness during the Hospital Phase. During the Active Phase, subjects were very regularly and actively followed up in order to maximize the detection of febrile and dengue episodes. During the Hospital Phase, subjects were followed up less actively and reporting was targeted on hospitalization. Dengue screening occurred in febrile subjects who required hospitalization. Regular contacts (e.g., phone calls, SMS, home visits, and school based surveillance) were scheduled during the Active phase, i.e. parents were reminded to take their child to the trial centre or health care centre in the event of acute febrile illness. There was an initial minimum frequency of one contact every week. Later on, the frequency could be changed. The method and frequency of contact could differ at each site and were detailed in the site-specific annexes. During the Hospital phase, this contact occurred at least every 3 months in addition surveillance of identified non-study healthcare sites.

Assessment methods for Virologically-confirmed (hospitalized) dengue cases

In the event of acute febrile illness (i.e. temperature ≥38°C on at least 2 consecutive days), two blood samples had to be collected.

The first blood sample had to be taken as soon as possible within 5 days of the onset of fever. Protocol mandatory testing included dengue immunoglobulin (Ig) M/IgG enzyme-linked immunosorbent assay (ELISA), dengue non-structural protein (NS) 1 ELISA antigen (Ag), dengue screen (DS) reverse transcriptase-polymerase chain reaction (RT-PCR), dengue serotype specific RT-PCR, haematocrit, platelet count, aspartate aminotransferase (AST) and alanine transaminase (ALT).

The second blood sample had to be taken during the convalescent phase of the disease (i.e. between 7 and 14 days after the acute sample). Testing had to include dengue IgM/IgG ELISA, haematocrit, platelet count, AST and ALT.

Similar methods had to be applied during the Hospital Phase for all acute hospitalized febrile cases.

Assessment methods related to the two endpoints 'severe dengue':

Based on the Investigator's judgment and on local standard of care, the Investigator performed key investigations using the list of characteristics predefined to identify severe cases of dengue. In all cases, haematocrit, platelet count, AST, ALT and a tourniquet test had to be performed. However, if a subject presented with other clinical signs of haemorrhage, the tourniquet test was not mandatory. For suspected dengue cases hospitalized in a non-study healthcare site, the Investigator had to ensure that these key biological parameters had been checked.

Sample size

In CYD14 study a total of 10,278 subjects were to be enrolled: 6852 subjects were to be included in the CYD Dengue Vaccine Group and 3426 subjects were to be included in the Control Group.

In CYD15 study a total of 20,875 subjects were planned to be enrolled: 13,917 subjects were to be included in the CYD Dengue Vaccine Group and 6958 subjects in the Control Group.

A subset of 2,000 subjects in each study (1,333 in the vaccine group and 667 in the control group) was evaluated for reactogenicity and immunogenicity.

Assuming an alpha=2.5% (one-sided hypothesis), a yearly incidence of symptomatic virologically-confirmed dengue cases of 1.3% for CYD14 and 0.64% for CYD15, an overall drop-out from the PPSE set of 20%, and a true VE of 70% after Dose 3, a total of 57 confirmed-dengue cases was expected during the 12-month active follow-up and this provides > 90% power to show a significant efficacy (lower bound of the 95% CI > 25%) using the exact method. In addition, the Applicant also considered that VE may have been 30% after the first dose and 50% after the second dose of CYD dengue vaccine.

The overall VE expected on the full analysis set for efficacy (FASE) population (with at least one dose of CYD dengue vaccine) at the end of the active follow-up was 55%, and therefore the expected FASE number of dengue cases was approximately 161 in study CYD14 and 155 in study CYD15 (occurring 28 days post-Dose 1 until the end of the Active Phase). Based on the planned sample size, there was at least an 87% power to conclude that the lower bound of the point estimate for VE on the FASE population is greater than 25%.

Randomisation

Each subject who met the inclusion criteria and none of the exclusion criteria and signed an ICF/AF was randomly assigned to one of two groups via an IVRS, according to a 2 to 1 ratio (2 subjects included in the CYD Dengue Vaccine Group for 1 subject included in the Control Group). Subjects randomized in the study during the first 2 months of enrolment were also randomized in the subset of subjects evaluated for the immunogenicity and reactogenicity in each country among the total number of subjects planned to be recruited during this period. The inclusion rate (per country) planned for the first 2 months was used to determine the ratio.

The randomizations (for allocation of the treatment group and inclusion in the subset) were performed with the permuted block method with stratification on sites/satellite and by age (i.e., 2 to 5 years, 6 to 11

years, and 12 to 14 years). This ensured that the balance per site and per age group between the number of subjects in the CYD Dengue Vaccine Group and the Control Group was respected as planned, with a 2 to 1 ratio.

A double randomization system was used for the doses randomization to ensure the blinding of the doses.

Statistical methods

Definition of study populations in each study and in the IEA

In general data were described by means of statistical characteristics (categorical variables: absolute and relative frequencies; numerical variables: mean, standard deviation, minimum and maximum) stratified for treatment group and time point (where applicable).

The following populations were defined for analyses in the individual studies:

Full Analysis Set for Efficacy (Other Efficacy Set in CYD23)	FASE (OES#1)	The FASE includes all subjects who received at least one injection. Used to assess efficacy from 28 days after the injection up to the end of the Active Phase or from the first injection to the end of the Active Phase
Other Efficacy Set	OES (OES#2 in CYD23)	OES includes subjects who received at least 2 injections of dengue or control vaccine. Used to assess efficacy from 28 days after the 2nd injection up to the end of the Active Phase.
Per-protocol analysis set for efficacy	PPSE	The PPSE includes all subjects who had no protocol deviations. Used for the analysis of VE from 28 days post-Dose 3 to the end of the Active Phase.
Modified Full Analysis Set for Efficacy	mFASE (FASE in CYD23)	The mFASE includes all subjects who received at least 3 injections, regardless of the per-protocol criteria. Used to assess efficacy from 28 days after the 3rd injection up to the end of the Active Phase.
Safety Analysis Set	SafAS	The SafAS includes all subjects who received at least one injection.
Full Analysis Set for Immunogenicity	FASI	The FASI is defined as the subjects of the immunogenicity and reactogenicity subset who received at least one injection and who had a blood sample drawn and a result available after this injection.

The primary efficacy analysis of the primary objective was performed on the PPSE, and was confirmed on the modified full analysis set for efficacy (mFASE) for CYD14 and CYD15 and on the FASE for CYD23. The FASE, the other efficacy analysis set (OEAS) and the mFASE were used for the secondary efficacy analyses, respectively after at least 1, 2 and 3 doses of vaccine/placebo. In the mFASE and the FASE, subjects were analysed according to the group to which they were randomized.

The other populations were used for the secondary and other efficacy analyses. Subjects were analysed according to the group to which they were randomized.

For the IEA, the same statistical populations from the individual studies were used as they are considered clinically similar. The PPSE was used to assess the efficacy for the primary objective only, in the PD3 period, i.e. from 28 days after the third injection to the end of the Active Phase. The mFASE was used to describe the efficacy after 3 injections, in the PD3 period. The FASE was used to describe the efficacy during the whole Active Phase period, i.e. from at least 1 injection (D0) to the end of the whole Active Phase. To describe efficacy according to the dengue immune status of subjects at baseline the Applicant used the FASE and mFASE populations restricted to subjects in the immunogenicity subset, in the PD3 and the whole Active Phase periods.

For FASE, mFASE and FASI, subjects were analysed according to the group to which they were randomized.

Statistical Method for Primary Efficacy Objective

To address the primary efficacy hypothesis (i.e. superiority of CYD vaccine group compared to control group), the following hypotheses were tested, using an alpha level of 2.5% (1-sided), on VE in preventing the occurrence of VCD cases after three doses:

- H0: VE ≤0 (CYD23) or ≤25% (CYD14 and CYD15)
- H1: VE >0 (CYD23) or >25% (CYD14 and CYD15)

The efficacy of the CYD dengue vaccine was estimated using the following formula:

VE = 100* [1- (PCYD / PP)] = 100* [1-((CCYD / NCYD) / (CP / NP))]

where: PCYD is the density incidence of dengue in the vaccine Group; PP is the density incidence of dengue in the Control Group; CCYD is the number of VCD cases in the vaccine Group in the PD3 period; NCYD is the number of person-year in the vaccine Group; CP is the number of VCD cases in the Control Group; NP is the number of person-years in the Control Group.

Person-years are the sum of individual units of time (years) for which the subjects contributed to the analysis. This is equal to the person-time at risk divided by 365.25.

For subjects with several episodes of dengue, only the first episode of VCD occurring more than 28 days after the third injection was included in the analysis of VE for the primary objective.

The statistical methodology was based on the use of the two-sided 95% confidence interval (CI) of the VE. The following statistics were provided: number of VCD cases, number of person-years at risk, density incidence and 95% CI, VE and 95% CI. CIs for the single proportion were calculated using the exact binomial method (Clopper-Person method, developed by Newcombe). CIs for VE were calculated using the exact method described by Breslow & Day. The VE of the CYD dengue vaccine was considered as significant if the lower bound of its 95% CI was greater than 25%. In addition, the Kaplan-Meier curves were drawn for some endpoints.

Statistical Method for Main Secondary and Other Efficacy Objectives

VE was assessed as for the primary objective over a different period of time or for another endpoint, depending on the objective. No hypotheses have been tested for secondary and other endpoints. The VE estimates in preventing symptomatic VCD cases were presented with their 95% Cis which were calculated using the exact method described by Breslow & Day.

The efficacy against at least 3 serotypes was calculated for each combination (serotypes 1-2-3, 1-2-4, 2-3-4, 1-3-4). Vaccine efficacy was evaluated on VCD cases, according to each dengue serotype after at

least 1, 2 and 3 doses. VE was defined as 1 minus the ratio of density incidences of each serotype in the CYD Dengue Vaccine Group over the density incidence of the Control Group.

In addition to VE, the density incidence and relative risk (RR) were calculated on subjects with VCD cases according to severity and according to serotype 28 days after each injection (to the end of the Active Phase) and from at least 1 injection (from D0) to the end of Active Phase. RR was defined as the ratio of density incidences in the Dengue Group to the Control Group.

Safety

The 95% CIs for percentages were calculated using the exact binomial distribution (Clopper-Pearson's method, quoted by Newcombe). Serious AESIs were also described using the same method. The number of subjects with serious dengue disease was summarized by country and time of onset.

Immunogenicity

Immunogenicity in the subset of subjects was assessed using the following parameters:

- GMT for each serotype (parental strains) before the first injection and 28 days after the second and the third injections, and 1 year after the third injection (other timepoints were also available);
- Geometric mean of the individual titre ratios (GMTR) for each serotype (parental strains) 28 days after the second and the third injection, based on the baseline neutralizing Ab titre;
- Number and percentage of subjects with dengue neutralizing Ab titre ≥ 10 (1/dil) (parental strains) 28 days after the second and the third injections and 1 year after the third injection;
- Number and percentage of subjects with dengue neutralizing Ab titre ≥10 (1/dil) against at least one, two, three, or the four dengue serotypes.
- Distribution of GMTs was described at each available time point.

The dengue serostatus at baseline was defined as seropositive if the PRNT50 titre was ≥10 (1/dil) against at least one serotype. This threshold represents the lower limit of quantification (LLOQ).

The 95% CIs were calculated using: The normal approximate method for GMTs and GMTRs, The exact binomial distribution for percentages (Clopper-Pearson's method, quoted by Newcombe).

Assuming that log10 transformation of the titres/ratios follows a normal distribution, first, the mean and 95% interval were calculated on log10 (titres/ratios) using the usual calculation for normal distribution, then antilog transformations were applied to the results of calculations, to compute GMTs/GMTRs and their 95% CIs.

Other specific immunogenicity analyses were performed according to the dengue and/or YF Ab levels at baseline and to the presence of a previous dengue infection.

<u>Statistical Methods for other endpoints analyses</u>

VE by serotype

VE against any and each of the four serotypes was presented with their 95% CI between each dose. Similar calculations were performed to assess VE estimates according to severity (WHO criteria and clinical criteria) for VCD cases.

VE against hospitalized dengue

Virologically-confirmed, hospitalized dengue cases due to each or any serotype occurring during the Active Phase were described. Stratified VE analyses and/or modelisation were performed to evaluate the relationship between the occurrence of dengue infection and some covariates, such as country, gender, age and presence of previous clinical history of YF/dengue infection or vaccination. For concerned subjects, adjustment on baseline dengue and/or YF Ab titre was used. Regression methods were used. As an exploratory analysis, a survival analysis approach (based on a time-to-event consideration) was used.

Virologically-confirmed, hospitalized dengue cases occurring during the Hospital Phase were described according to severity.

Serology of dengue cases

The serological profile of suspected dengue cases were based on IgG and IgM ELISA results. Descriptive statistics were used.

Viremia

In dengue cases confirmed by DS RT-PCR, the viremia level of acute blood sample was summarized. This was done for each serotype and according to severity.

Evaluation of Relationship between Neutralizing Ab Levels and efficacy

The GMTs of subjects with VCD cases 28 days post-dose 3 were compared with the GMTs of subjects included in the immunogenicity subset without VCD cases (since inclusion), per serotype and for any serotype. For the determination of GMTs per serotype, VCD cases were defined as serotype-specific.

A logistic regression and a Log-Scale Logit model were used to evaluate the association between the Ab level and dengue occurrence.

Statistical Methods for Exploratory Analyses

Time-to-Event Analysis

As an exploratory analysis of the primary endpoint, a survival analysis approach (based on a time-to-event consideration) was used. For each treatment group, a Kaplan-Meier curve along with the log-rank test comparing the 2 curves was determined. The endpoint was then the time (in years) that the subjects were exposed to or at risk of developing a dengue fever from 28 days post-Dose 3 or from D0. The VE and its 95% CI were also obtained using a Cox hazards regression model with vaccine group as covariate.

Cox regression assumes proportional hazards throughout the follow-up period. This assumption was checked by a test based on the scaled Schoenfeld residuals. The Kaplan-Meier curves with log-rank test and the Cox regression were also provided by serotype.

Covariate Adjustment and Subgroup Analyses

Vaccine Efficacy

Stratified VE analyses 28 days after the third dose and during the whole Active Phase were performed to evaluate the relationship between the occurrence of dengue infection (against any and each serotype) and the following covariates: country; age group and age (as a continuous variable); gender, and presence of the following reported at baseline: previous clinical history of dengue infection, previous clinical history of YF infection, previous YF vaccination, previous clinical history of dengue and/or YF infection/vaccination.

A Cox proportional hazards regression with the covariates and the vaccine group was computed. The interactions between the covariates and the vaccine group were tested. If there was not significant at the level of 15% (threshold arbitrarily chosen to not exclude covariates that could potentially have a significant impact on the endpoint), the models were fitted without the interaction terms.

The relative risk (RR) analyses on symptomatic virologically-confirmed dengue cases were performed for the immunogenicity subset according to the FV status (dengue and YF) at baseline, based on serological results obtained at D0:

- dengue status at baseline
- YF status at baseline
- FV (dengue and/or YF) status at baseline

Immunogenicity and Reactogenicity

Descriptive exploratory analyses for immunogenicity and reactogenicity were also performed according to the same covariates.

Handling of dropouts or missing data

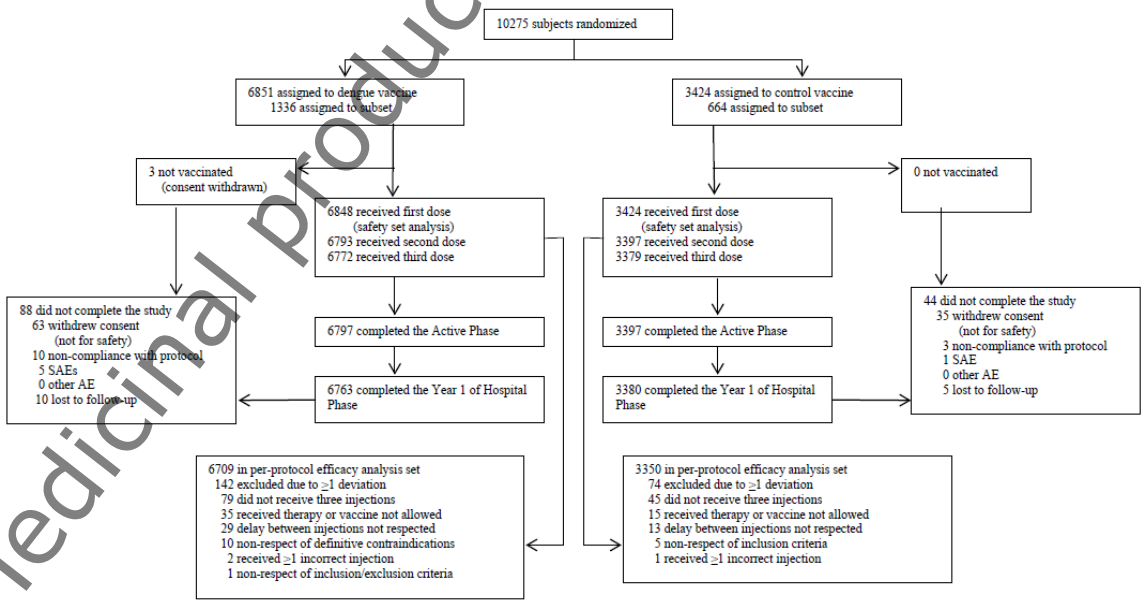
Subject exclusion from efficacy analyses was determined by type of missing data and described in details in the SAP. Sensitivity analyses were planned in the SAP. Missing efficacy data were not imputed. No test or search for outliers was performed.

Results

Participant flows

Study CYD14

Figure 6: Subject disposition for study CYD14



10,275 subjects were randomized out of the 10,278 planned subjects (3 subjects were randomized twice). Of these subjects, 2000 subjects (1333 in the CYD Dengue Vaccine Group and 667 in the Control Group) were randomized to the reactogenicity and immunogenicity subset. Out of the subjects enrolled and randomized (6851 subjects in the CYD Dengue Vaccine Group and 3424 subjects in the Control

Group), 10,274 were eligible at V01. The reason for non-eligibility was that one subject in the CYD Vaccine Group received a vaccine in the 4 weeks preceding the first trial vaccination (exclusion criterion 14).

Subjects were randomized and attended Visit 01 within the period 03 June 2011 to 01 December 2011.

A total of 6771 subjects (98.8%) received 3 doses of the CYD dengue vaccine as per protocol. The number and percentage of subjects in the CYD Dengue Vaccine Group who did not receive the 3-dose schedule was as follows:

- At V01, 3 (0.0%) subjects did not receive the first injection.
- At V03, 85 (0.8%) subjects did not receive two injections
- At V05, 124 (1.2%) subjects did not receive the three injections

A high percentage of subjects completed the Active Phase (99.2%).

Study CYD15

Table 4: Subject disposition for study CYD15

	CYD Dengue vaccine N	Control N	All N
Planned sample size	13,917	6,958	20,875
Randomized subjects	13,920	6,949	20,869
Subset of Subjects	1,334	666	2,000
Subjects who completed the Active Phase	13,281	6,640	19,921
Discontinued subjects	639	309	948
SAE	7	9	16
Other AE	3	0	3
Non-compliance with protocol	43	14	57
Lost to follow-up	106	46	152
Voluntary withdrawal not for AE	480	240	720
Subjects who completed the first year of Hospital Phase	13,074	6545	19,921
Discontinued subjects	846	404	1250
SAE	12	10	22
Other AE	3	0	3
Non-compliance with protocol	48	18	66
Lost to follow-up	189	83	272
Voluntary withdrawal not for AE	594	293	887
Subjects in Per Protocol Efficacy Set*	12,573	6,261	18,834

* Population of analysis used for the evaluation of the primary objective. All subjects presenting with at least one deviation as defined per protocol were excluded from this set.

A total of 19,921 (95.5%) subjects (13,281 [95.4%] in the CYD Dengue Vaccine Group and 6640 [95.6%] in the Control Group) completed the Active Phase. Although the protocol planned that subjects who discontinued from vaccination period had to continue surveillance for dengue until the end of the study, a proportion of subjects (4.5%) could not been re-contacted at V07 and therefore did not complete the Active Phase.

Reasons for discontinuation during the Active Phase were mostly voluntarily withdrawals not due to an AE, in 3.5% of subjects (3.4% in the CYD Dengue Vaccine Group and 3.5% in the Control Group) and "lost to follow-up" in 0.7% of subjects (0.8% in the CYD Dengue Vaccine Group and 0.7% in the Control Group). Other reasons for discontinuation were non-compliance with study protocol in 0.3% of the

subjects (0.3% of subjects in the CYD Dengue Vaccine Group and 0.2% of subjects in the Control Group, and "occurrence of a SAE" in 0.1% of subjects (0.1% in the 2 groups).

For both trials the percentages of subjects in each of the subcategories for discontinuation (voluntarily withdrawals, lost-to-follow-up, etc.) were similar in the vaccine and placebo groups.

Conduct of the studies

For the two pivotal trials, the initial version of the protocol, Version 1.0, was issued on 12 March 2010. There were four protocol amendments in total in both studies. The first was approved prior to the start of the trial to ensure compliance with WHO guidelines and consistency with the IDMC's definitions. In amendment 2, CYD14 and CYD15 clinical trial protocols were modified to clarify the stratification by age; 2 to 5 years, 6 to 11 years and 12 to 14 in CYD14 and 9 to 11 years and 12 to 16 years to balance randomization per age group in CYD15. In the third amendment, the results of CYD23 trial were communicated to all Ethics Committees of both pivotal studies and several modifications were implemented taking into account these results. As a consequence, other objectives were added, the hospital Phase was extended by 2 years to allow a 5 year follow-up period after the last vaccination. In addition the Simplexa dengue RT-PCR replaced the WT dengue RT-PCR because it is able to qualitatively detect strains not detected by the WT RT-PCR. The fourth amendment (dated 25 Jan 2015) was implemented to reactivate surveillance of all symptomatic cases during long term follow up, the so-called "surveillance expansion phase ('SEP').

During the Active Phase, there was a low proportion of protocol deviations, which were balanced between groups, and a low proportion of lost to follow up (not contacted at 24 months) overall (<1%) and in each country (<4%). Lost to follow up frequency was low in the Hospital Phase as well, but protocol deviations were more frequent during this Phase (>5%) and varied by country, but was balanced between groups.

At the time of MAA submission the CYD14 and CYD15 trials were ongoing. The CYD14 trial was completed on November 21st 2017. CYD15 was completed on March 5th 2018.

Baseline data

CYD14

All subjects but one were Asian. Overall, there were similar percentages of female (51.5%) and male subjects (48.5%); these proportions were similar in each treatment group. The mean age at enrolment was 8.8 years in both treatment groups. The number of subjects per age groups is indicated in the table below (PPSE).

Age group: n (%)	CYD vaccine group (N=6709)	Control group (N=3350)	All (N=10,059)
2 to 5 years	1615 (24.1%)	795 (23.7%)	2410 (24%)
6 to 11 years	3567 (53.2%)	1793 (53.5%)	5360 (53.3%)
12 to 14 years	1527 (22.8%)	762 (22.7%)	2289 (22.8%)

The demographic characteristics were also comparable across countries, including the age group distribution with approximately more than half of the subjects in the 6 to 11 years age group in each country. The distribution by country is summarized in Table 5 for randomized subjects and for the subjects included in the subset.

Table 5: Subjects by country and randomised treatment group – Randomised subjects and randomised subjects included in the subset

	CYD Dengue Vaccine Group (N=6851)		Control Group (N=3424)		All Subjects (N=10275)	
	n	n subset	n	n subset	n	n subset
Indonesia	1246	234	624	116	1870	350
Malaysia	937	204	464	96	1401	300
Philippines	2335	402	1166	200	3501	602
Thailand	778	225	392	116	1170	341
Vietnam	1555	271	778	136	2333	407

n: number of subjects fulfilling the item listed

CYD15

Distribution between genders was equal in all groups. The mean age at enrolment was 12.4 years in both treatment groups. The number of subjects per age groups (n(%)) is indicated below (PPSE):

- 9 to 11 years: 5770 (45.9) in the vaccine group and 2860 (45.7) in the control group (8630, 45.8 in total).
- 12 to 16 years: 6803 (54.1) in the vaccine group and 3401 (54.3) in the control group (10,204, 54.2 in total).

The ethnic origin of the subjects was American Indian (16.2%), Caucasian (8.0%), Black (3.1%) but most of subjects reported being Hispanic of mixed ethnic origins, classified as "Other" (72.6%). Demographic characteristics were very similar in the 2 treatment groups.

The distribution by country and treatment group of the overall of subjects randomized in the study and the subset is summarized in Table 6. Approximately half of the subjects were included in Colombia (9743 subjects out of 20,869). This higher percentage of subjects was recruited in Colombia because this was the country with a history of more sustained circulation of the 4 serotypes in the years prior to the onset of the study. Brazil and Mexico included respectively 3548 and 3464 subjects. Honduras and Puerto Rico recruited respectively 2799 and 1315 subjects.

Table 6: Country distribution and randomised treatment group in the overall population and in the overall population and in the immunogenicity and reactogenicity subset – Randomised subjects

	CYD Dengue Vaccine Group (N=13920)		Control Group (N=6949)		All Subjects (N=20869)	
	Overall population n	Subset n	Overall population n	Subset n	Overall population n	Subset n
All	13920	1334	6949	666	20869	2000
Brazil	2370	202	1178	98	3548	300
Colombia	6497	613	3246	308	9743	921
Honduras	1866	200	933	100	2799	300
Mexico	2312	219	1152	108	3464	327
Puerto Rico	875	100	440	52	1315	152

n: number of subjects fulfilling the item listed

Although the percentage of Caucasians (the main ethnic group in Europe) included in the trials is low, there is no reason to think that the vaccine will behave differently in different ethnic groups, and thus it is considered that the immunogenicity and efficacy data obtained from these trials can be extrapolated to the EU population.

Baseline Status for flavivirus, Dengue and Yellow Fever (FASI)

CYD14

Data on baseline dengue and Japanese encephalitis (JE) antibody (Ab) levels were obtained in the subset. In the FASI (n=1983), 67.6% were dengue immune to at least one serotype (neutralizing Ab response ≥10 (1/dil) using Dengue PRNT50) and 42% to all 4 serotypes in the vaccine group, similarly to the control group. Overall 52.6% were Japanese encephalitis immune (n=1043) at baseline. A total of 78.2% of the subjects were flavivirus (FV) (JE and/or dengue) immune at baseline (n=1551). These proportions were well balanced between both groups (Vaccine and Control). Subjects from the Vaccine and the Control Groups were also comparable in terms of GMTs at baseline.

The proportion of dengue immune subjects at baseline varied with country: from 47.8% of dengue immune subjects at baseline in Malaysia to 80.8% in Indonesia. Important differences in terms of JE immune status at baseline by country were also observed, which may reflect the diversity in JE vaccination program since only 45.5% of subjects were JE immune in the Philippines whereas no JE immunization program is implemented except in private market. However these results are difficult to interpret due to cross-reactivity with JE and other flaviviruses.

The proportion of dengue immune subjects at baseline increased with age: from 51.3% for the 2 to 5 years age group to 81.0% for the 12 to 14 years age group (Table 7).

Table 7: Flavivirus, dengue and Japanese Encephalitis immune subjects, by age group and randomized group - Full Analysis Set for Immunogenicity - CYD14

		CYD Dengue Vaccine Group	Control Group	All Subjects
Age group		(N=1323) n (%)	(N=660) n (%)	(N=1983) n (%)
2 to 5 years	Baseline flavivirus status	313 (68.8)	140 (62.8)	453 (66.8)
	Baseline dengue status	243 (53.4)	105 (47.1)	348 (51.3)
	Baseline JE status	185 (40.7)	83 (37.2)	268 (39.5)
6 to 11 years	Baseline flavivirus status	381 (81.4)	196 (82.4)	577 (81.7)
	Baseline dengue status	333 (71.2)	174 (73.1)	507 (71.8)
	Baseline JE status	249 (53.2)	125 (52.5)	374 (53.0)
12 to 14 years	Baseline flavivirus status	348 (87.0)	173 (86.9)	521 (87.0)
	Baseline dengue status	320 (80.0)	165 (82.9)	485 (81.0)
	Baseline JE status	268 (67.0)	133 (66.8)	401 (66.9)

n: number of subjects fulfilling the item listed

During the Active Phase, all 4 serotypes were circulating in the 5 countries although serotypes distribution varied according to country. The density incidence of virologically-confirmed dengue (VCD) in the Control Group was 4.7 (95%CI: 4.2; 5.2) per 100 person-year at risk during the Active Phase. During the Active Phase, the density incidence varied across serotypes with 1.9%, 1.1%, 0.6%, and 1.0% in the Control Group for serotypes 1, 2, 3, and 4, respectively. The incidence density of VCD due to any serotype in the Control Group during the Active Phase decreased with age. In the immunogenicity subset, the density incidence of VCD in the Control Group was 4.0 (3.0; 5.2) per 100 person-year at risk during the Active Phase.

CYD15

Overall, 86.0% of the subjects were Flavivirus-seropositive at baseline and 79.4% of the subjects were dengue-seropositive at baseline. A slight imbalance between vaccine and control group is observed as

higher seropositivity rates occur in the vaccine group (86.7% and 80.6%, resp.) compared to the control group (84.4% and 77.0%, resp.).

Baseline Flavivirus-seropositivity rates varied by country and were lower in Mexico and Puerto Rico (~59%) compared to the 3 other countries (at least 86.7%). Baseline dengue-seropositivity rates varied by country and were higher in Colombia (92.2%) and Honduras (85.7%) compared to the other countries such as Mexico (53.1%) and Puerto Rico (56.2%).

Overall, 79.7% of the subjects were YF-seropositive at baseline. Baseline YF-seropositivity rates varied by country and were higher in Colombia (96.0%), Brazil (82.1%) and Honduras (79.4%) compared to the other countries such as Mexico (47.5%) and Puerto Rico (45.2%).

Most subjects were positive for both dengue and YF; therefore, the percentage of subjects positive to both dengue and YF are slightly lower than those of the individual viruses.

As expected higher seropositivity rates to flaviviruses were observed in older subjects (Table 8):

Table 8: Baseline flavivirus, dengue and Yellow Fever seropositivity, by age and randomized group - Full Analysis Set for Immunogenicity – CYD15

Age group	Seropositivity at baseline	CYD Dengue Vaccine Group (N=1301) n (%)	Control Group (N=643) n (%)	All Subjects (N=1944) n (%)
9 to 11 years	Flavivirus	547 (85.1)	264 (81.5)	811 (83.9)
	Dengue	494 (76.8)	230 (71.0)	724 (74.9)
	Yellow Fever	503 (78.2)	243 (75.0)	746 (77.1)
	Dengue and Yellow Fever	450 (70.0)	209 (64.5)	659 (68.1)
12 to 16 years	Flavivirus	581 (88.3)	279 (87.5)	860 (88.0)
	Dengue	554 (84.2)	265 (83.1)	819 (83.8)
	Yellow Fever	539 (81.9)	265 (83.1)	804 (82.3)
	Dengue and Yellow Fever	512 (77.8)	251 (78.7)	763 (78.1)

n: number of subjects fulfilling the item listed
Flavivirus- seropositive subjects at baseline are defined as subjects with baseline Yellow Fever and/or at least one dengue serotype >= 10 (1/dil).
Dengue-seropositive subjects at baseline are defined as subjects with titers >= 10 (l/dil) against at least one dengue serotype at baseline.
Yellow Fever-seropositive subjects at baseline are defined as subjects with Yellow Fever titer >= 10 (l/dil) at baseline.
Dengue- and Yellow Fever -seropositive subjects at baseline are defined as subjects with titers >= 10 (l/dil) against at least one dengue serotype and against Yellow Fever at baseline.

Numbers analysed

Table 9: Population of analysis for CYD14

	Vaccine Group	Control group
Populations of analysis		
Enrolled and randomized	6851	3424
Full Analysis Set for Efficacy (FASE)	6848	3424
Per-Protocol Set for Efficacy (PPSE)	6709	3350

Modified Full Analysis Set for Efficacy (mFASE)	6772	3379
Other Efficacy Analysis Set (OES)	6793	3397
Safety Analysis Set (SafAS)	6848	3424
Full Analysis Set for Immunogenicity (FASI)	1323	660
Hospital phase	6778	3387
Populations excluded from the analyses		
Subjects with protocol deviations during the Active Phase	142 (2.0%)	74 (2.2%)
Subjects who did not receive all three doses	79 (1.1%)	45 (1.3%)

Table 10: Efficacy analysis sets by randomized group – Randomized Subjects – CYD15

	CYD Dengue Vaccine Group (N=13920) n (%)	Control Group (N=6949) n (%)	All (N=20869) n (%)
Per-Protocol Set for Efficacy	**12573 (90.3)**	**6261 (90.1)**	**18834 (90.2)**
Full Analysis Set for Efficacy	**13914 (100.0)**	**6940 (99.9)**	**20854 (99.9)**
Not injected	4 (0.0)	9 (0.1)	13 (0.1)
Severe non-compliance with GCPs	2 (0.0)	0 (0.0)	2 (0.0)
Modified Full Analysis Set for Efficacy	**13288 (95.5)**	**6643 (95.6)**	**19931 (95.5)**
Not received 3 injections	630 (4.5)	306 (4.4)	936 (4.5)
Severe non-compliance with GCPs	2 (0.0)	0 (0.0)	2 (0.0)
Other Efficacy Analysis Set	**13506 (97.0)**	**6765 (97.4)**	**20271 (97.1)**
Not received 2 injections	412 (3.0)	184 (2.6)	596 (2.9)
Severe non-compliance with GCPs	2 (0.0)	0 (0.0)	2 (0.0)

n: number of subjects fulfilling the item listed
Note: a subject may be associated with more than one deviation
Subjects who received a vaccine not authorized for use are reported under the deviation "Administration of the vaccine was not done as per protocol (site and route)".

Table 11: Immunogenicity analysis set by randomized group – Randomized Subjects Included in the Subset – CYD15

	CYD Dengue Vaccine Group (N=1334) n (%)	Control Group (N=666) n (%)	All (N=2000) n (%)
Full Analysis Set for Immunogenicity	**1301 (97.5)**	**643 (96.5)**	**1944 (97.2)**
Not injected	1 (0.1)	2 (0.3)	3 (0.2)
Not blood sample drawn or no result available after injection	32 (2.4)	21 (3.2)	53 (2.7)
Severe non-compliance with GCPs	0 (0.0)	0 (0.0)	0 (0.0)
Per-Protocol Set for Immunogenicity post-Inj 2	**1206 (90.4)**	**584 (87.7)**	**1790 (89.5)**
Per-Protocol Set for Immunogenicity post-Inj 3	**1216 (91.2)**	**580 (87.1)**	**1796 (89.8)**

n: number of subjects fulfilling the item listed
Note: a subject may be associated with more than one deviation
Subjects who received a vaccine not authorized for use are reported under the deviation "Administration of the vaccine was not done as per protocol (site and route)".
Subject who was injected before being randomized is reported under the deviation "Received at least one dose of a product other than the one randomized to receive" even if he received the correct treatment.

Outcomes and estimation – EFFICACY

A summary of estimates of efficacy for individual trials CYD14 and CYD15 is included respectively in Table 13 and Table 14. For study CYD23/CYD57 see below. The results of the Integrated Efficacy Analysis including data from the 3 trials CYD14/CYD15/CYD23 are described in Section 2.5.3 Analyses across trials.

CYD23 results

A total of 4002 subjects were evaluated at a single site in Thailand. A total of 78 VCD cases occurring from 28 days post-injection 3 to the end of the Active Phase were observed in 77 subjects and were used to calculate VE. During PD3, VE in the PPSE was 30.2% (95% CI: -13.4; 56.6), and differed by serotype. The primary estimate of VE was lower than anticipated and was not significant. VE was assessed against any serotype and by serotype after at least one injection: VE against any serotype was 34.9% and significant (95% CI 6.7; 54.3), VE against dengue serotypes 1, 3 and 4 was respectively 61.2%, 81.9%, and 90.0%, and statistically superior to 0 after at least 1 injection. VE after at least one injection against dengue serotype 2 was not demonstrated and not statistically superior to 0 (3.5%).

The overall VE estimate did not reach levels of statistical significance since the lower bound of the 95% CI was less than 0. This result was driven primarily by the finding that most of the serotypes identified were serotype 2 (32 VCD cases in the dengue group and 19 VCD cases in the control group were due to serotype 2). Nevertheless, clinical proof of concept was considered demonstrated given the measurable efficacy.

After the third injection, 91.5% of subjects were seropositive against all 4 serotypes. One year after the third injection, GMTs were 2 to 4 times higher than baseline GMTs. A trend toward higher GMTs was noted in older subjects. Baseline data observed in both treatment groups are consistent with the local epidemiological situation of dengue in Thailand. The safety profile of the CYD dengue vaccine was satisfactory, with decreasing systemic reactogenicity after subsequent injections.

Results of CYD57 up to Year 2 of the hospithal phase

Long-term follow-up of safety and hospitalized dengue cases is being evaluated through Study CYD57. The safety data are discussed in the Clinical Safety part of this report, and are mostly pooled with CYD14 and CYD15 safety data.

During the first 2 years of Hospital Surveillance in CYD57, there were 7 hospitalized VCD assessed as clinically severe by the IDMC: 5 in the Dengue Group and 2 in the Control Group, taking into account the randomization ratio 2:1 between the Dengue Group and Control Group. The analysis by serotypes showed that the serotype the most represented was serotype 1 (4 out of 5 cases in the Dengue Group).

During the first year of Hospital Surveillance, 4 hospitalized SVCD cases were reported in the Dengue Group and no case was reported in the Control Group; resulting in an annual incidence of hospitalized SVCD was low in both groups (0.2% in the Dengue Group and 0.0% in the Control Group). Serotype 1 was the predominant serotype during the first year of Hospital Surveillance.

During the second year of Hospital Surveillance, 1 hospitalized SVCD case was reported in the Dengue Group and 2 SVCD cases were reported in the Control Group, resulting in an annual incidence rate of hospitalized SVCD of < 0.1% in Dengue Group and of 0.2% in the Control Group with a RR value that was < 1, but was not statistically significant (RR: 0.251 [95% CI: 0.00; 4.82]). There was no predominant serotype during the second year of Hospital Surveillance.

There was no evidence of excess of hospitalized SVCD cases in the Dengue Group compared to the Control Group up to the second year of the Hospital Surveillance in the CYD57 safety follow-up.

Most SVCD cases were rated DHF Grade I and II according to WHO classification. The most frequently observed clinical symptoms were plasma leakage, thrombocytopenia, and haemorrhagic signs. Two subjects in the Dengue Group reported clinical shock (identified using the Sponsor specific algorithm and confirmed by the IDMC) during the first year of Hospital Surveillance. Both cases of clinical shock were rated DHF Grade III according to WHO classification. The first case occurred in a 9-year-old (at the time of the event) female who showed spontaneous bleeding (hematemesis), platelet count of 9000/µL and signs of plasma leakage. The second case occurred in a 7-year-old female (at the time of the event) who had a positive Tourniquet test, a platelet count of 24,000/µL and presented signs of circulatory failure (rapid and weak pulse). All subjects with SVCD recovered with supportive medical treatment.

Table 12: Incidence of hospitalized SVCD (as per IDMC) due to any serotype collected during the Hospital Surveillance/Phase - All Subjects and per age group– Safety Analysis Set CYD57

		CYD Dengue Vaccine Group					Control Group					Relative Risk	
Study	Time period Age at inclusion	Cases	M	Annual Incidence Rate	(95% CI)	n Episodes	Cases	M	Annual Incidence Rate	(95% CI)	n Episodes	RR	(95% CI)
CYD57	Hospital Surveillance Year 1 (all cases)	4	2133	0.2	(0.1; 0.5)	4	0	1070	0.0	(0.0; 0.4)	0	NC	(NC)
	6-11 years	2	1740	0.1	(0.0; 0.5)	2	0	878	0.0	(0.0; 0.5)	0	NC	(NC)
	4-5 years	2	393	0.6	(0.1; 2.0)	2	0	192	0.0	(0.0; 2.1)	0	NC	(NC)
	≥9 years (9-11 yo)	0	794	0.0	(0.0; 0.5)	0	0	406	0.0	(0.0; 1.0)	0	NC	(NC)
	< 9 years (4-8 yo)	4	1339	0.3	(0.1; 0.8)	4	0	664	0.0	(0.0; 0.6)	0	NC	(NC)
	Hospital Surveillance Year 2 (all cases)	1	2133	<0.1	(0.0; 0.3)	1	2	1070	0.2	(0.0; 0.7)	2	0.251	(0.00; 4.82)
	6-11 years	0	1740	0.0	(0.0; 0.2)	0	1	878	0.1	(0.0; 0.6)	1	0.000	(0.00; 19.68)
	4-5 years	1	393	0.3	(0.0; 1.4)	1	1	192	0.5	(0.0; 2.9)	1	0.489	(0.01; 38.35)
	≥9 years (9-11 yo)	0	794	0.0	(0.0; 0.5)	0	0	406	0.0	(0.0; 0.9)	0	NC	(NC)
	< 9 years (4-8 yo)	1	1339	<0.1	(0.0; 0.4)	1	2	664	0.3	(0.0; 1.1)	2	0.248	(0.00, 4.76)

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 13: Summary of Efficacy for trial CYD14

Study Title: Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 14 years in Asia			
Study identifier	**CYD14**		
	Clinical Study Report Up to 24 months post-Injection 3 (Year 1 Hospital Phase)		
Design	Randomized, observer-blind, placebo-controlled, multi-center, Phase III trial in 5 endemic countries. The trial enrolled 10,278 healthy children and was conducted at 11 sites across Thailand, Malaysia, Indonesia, Philippines and Vietnam.		
	Duration of Active phase: Duration of Hospital phase: Duration of Extension phase:		03 June 2011 (FVFS) – 16 December 2013 (LVLS) (ca. 25 months per subject) 17 June 2013 – ongoing (ca. 47 months per subject) 2015 – 2017 (LVLS: 21Nov2017)
Hypothesis	Superiority		
Treatments groups	CYD Dengue Vaccine group		Subjects received 3 doses (0, 6 and 12 months) of CYD Dengue Vaccine with an efficacy follow-up of 13 months after Dose 3 and a follow-up for hospitalized dengue cases of 60 months after Dose 3. **N = 6852**
	Control Group		Subjects received 3 vaccinations (0, 6 and 12 months) of saline solution (NaCl 0.9%) with an efficacy follow-up of 13 months after Dose 3 and a follow-up for hospitalized dengue cases of 60 months after Dose 3. **N = 3426**
Endpoints and definitions	Primary endpoint	Efficacy	Symptomatic virologically-confirmed dengue cases occurring > 28 days after Dose 3 (during the Active Phase) and defined as: • Acute febrile illness (temperature ⩾ 38°C on at least 2 consecutive days) • Virologically-confirmed by dengue RT-PCR and/or dengue NS1 ELISA Ag test
	Secondary endpoint	Efficacy	Symptomatic virologically-confirmed dengue cases **after the third dose to the end of the Active Phase**: a. due to at least 3 serotypes b. due to each of the 4 serotypes
			Symptomatic virologically-confirmed dengue cases **after at least 1 dose**: a. due to any of the 4 serotypes b. due to at least 3 serotypes c. due to each of the 4 serotypes
			Symptomatic virologically-confirmed dengue cases **after 2 doses**: a. due to any of the 4 serotypes b. due to at least 3 serotypes c. due to each of the 4 serotypes

	Secondary endpoint	Safety	**Occurrence of SAEs**, including serious AESIs, in all subjects throughout the entire study The following serious AESIs were considered: • Serious hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination • Serious viscerotropic disease occurring in all subjects within 30 days after the vaccination • Serious neurotropic disease occurring in all subjects within 30 days after the vaccination • **Serious dengue disease**, as diagnosed by the Investigator, occurring in all subjects at any time during the study (protocol 6.0). This was replaced by the following in protocol version 7.0: To describe the occurrence of hospitalized virologically-confirmed dengue cases and the occurrence of severe (clinically-severe or as per WHO criteria) virologically-confirmed dengue cases, throughout the SEP and throughout the trial (from D0 until the end of the trial)
	Secondary endpoint in a subset (N = 2000)	Immunogenicity	Neutralizing Ab level against each of the four parental dengue virus strains of CYD dengue vaccine constructs (and potentially against recently isolated strains) measured at baseline, after Dose 2, after Dose 3, and 1, 2, 3, 4 and 5 years after Dose 3 (dengue neutralization assay). In addition, baseline neutralizing Abs against Japanese Encephalitis were described.
	Secondary endpoint in a subset (N = 2000)	Reactogenicity	• unsolicited systemic AEs reported in the 30 minutes after each dose • injection site reactions occurring up to 7 days after each dose • solicited systemic reactions occurring up to 14 days after each dose • unsolicited (spontaneously reported) AEs up to 28 days after each dose • non-serious AESIs occurring up to 7 days after each dose.
	Other endpoint	Efficacy	Dengue cases during the active phase • virologically confirmed dengue that meets 1997 WHO criteria for DHF due to any of the four serotypes after each dose • clinically-severe virologically-confirmed dengue cases due to any of the four serotypes after each dose • virologically-confirmed hospitalized dengue cases due to each or any of the 4 serotypes • Symptomatic virologically-confirmed dengue cases due to each or any of the 4 serotypes between each doses

	Other endpoint	Efficacy	Dengue cases during the hospital phase: virologically-confirmed hospitalized dengue cases (protocol version 6.0). This was replaced by the following in protocol version 7.0: Detection of dengue cases during the Surveillance Expansion period 1) To describe the efficacy of CYD dengue vaccine in preventing symptomatic virologically-confirmed dengue cases, hospitalized cases and severe cases, due to each or any of the 4 serotypes after at least 1 dose. 2) to assess the risk factors associated with hospitalization and severity of virologically-confirmed dengue cases, during the SEP.
	Other endpoint	Immunogenicity	Serological profile of suspected dengue cases
	Other endpoint	Safety	Dengue viremia • WT dengue strain viremia level in acute samples of virologically confirmed dengue cases
	Other endpoint	Efficacy Immunogenicity	Relationship Between Neutralizing Ab Level and Vaccine Efficacy • relationship between post-Dose 3 neutralizing Ab level and the subsequent occurrence of symptomatic dengue cases
	Other endpoint		Medical and Non-Medical Resource Utilization Related to Dengue Disease • level of medical and non-medical resource utilization linked to hospitalized and ambulatory confirmed dengue cases
Database lock	Database lock for the Active Phase: 19 March 2014 Database lock for the Hospital Phase Year 1: 19 February 2015		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Per Protocol Analysis Set for Efficacy (PPSE) Data collected from 28 days after the third dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.			
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	6709 (97.9%)	3350 (97.8%)	
	Symptomatic virologically-confirmed dengue cases post-dose 3 due to any of the 4 serotypes	117	133	
	Person-years at risk	6525	3227	
	Density incidence (per 100 person-year at risk)	1.8	4.1	

	95% CI	1.5; 2.1	3.5; 4.9	
Effect estimate per comparison	Primary endpoint	Comparison groups		Vaccine vs. Control
		Vaccine efficacy		56.5%
		95%CI		43.8; 66.4
		P-value		The vaccine efficacy is considered as significant if the lower bound of its 95% CI (exact method described by Breslow & Day) is greater than 25%.
Analysis description	**Secondary analysis (1): Efficacy against each serotype after 3 doses**			
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Modified Full Analysis Set for Efficacy (mFASE); n=6772 (98.8%) and n=3379 (98.7%). Data collected from 28 days after the third dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.			
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases post-dose 3, due to each of the 4 serotypes	Comparison groups		Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4		 50.0% 35.0% 78.4% 75.3%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4		 24.6; 66.8 -9.2; 61.0 52.9; 90.8 54.5; 87.0
Analysis description	**Secondary analysis (2): Efficacy after at least 1 dose**			
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE); n=6848 (100.0%) and n=3424 (100.0%). Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination			
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups		Vaccine vs. Control
		Vaccine efficacy:		54.8%
		95%CI:		46.8; 61.7
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to each of the 4 serotypes	Comparison groups		Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4		 54.5% 34.7% 65.2% 72.4%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4		 40.9; 64.9 10.4; 52.3 43.3; 78.9 58.8; 81.7
Analysis description	**Secondary analysis (3): Efficacy after 2 doses**			

Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Other Efficacy Analysis Set (OEAS); n=6793 (99.2%) and n=3397 (99.2%). Data collected from 28 days after the second dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after 2 doses, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy:	57.9%
		95%CI:	49.0; 65.2
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after 2 doses, due to each of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 56.7% 37.9% 70.7% 73.6%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 40.9; 68.3 10.1; 56.9 47.7; 84.0 58.7; 83.3
Analysis description	**Secondary analysis (4): Seropositivity against each serotype**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1323 (19.3%) and n=660 (19.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titres $\geqslant$ 10 (1/dil)) against each serotype, **baseline**	Comparison groups	Vaccine vs. Control
		% seropositive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 52.0% vs. 51.3% 58.0% vs. 59.3% 56.8% vs. 59.4% 51.6% vs. 50.7%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (49.3;54.8) vs. (47.4;55.2) (55.3;60.7) vs. (55.5;63.1) (54.1;59.6) vs. (55.5;63.2) (48.9;54.4) vs. (46.8;54.6)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titres $\geqslant$ 10 (1/dil)) against each serotype, **post-Dose 3**	Comparison groups	Vaccine vs. Control
		% seropositive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 94.0% vs. 55.4% 98.7% vs. 61.8% 97.0% vs. 61.0% 97.0% vs. 53.9%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (92.6;95.2) vs. (51.5;59.2) (97.9;99.2) vs. (58.0;65.5) (95.9;97.8) vs. (57.2;64.8) (95.9;97.8) vs.(50.0;57.7)
Effect estimate per comparison	Seropositive subjects	Comparison groups	Vaccine vs. Control

	(neutralizing Ab titers ⩾ 10 (1/dil)) against each serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	% seropositive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 74.7% vs. 58.4% 88.2% vs. 67.4% 87.5% vs. 64.6% 83.1% vs. 54.5%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (72.2;77.1) vs. (54.5;62.3) (86.3;89.9) vs. (63.6;71.0) (85.5;89.3) vs. (60.7;68.3) (80.9;85.1) vs. (50.6;58.5)
Analysis description	**Secondary analysis (5): Seropositivity against at least one serotype**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1323 (19.3%) and n=660 (19.3%). Data collected from Day 0 to the end of the Active Phase i.e., ., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **baseline**	Comparison groups	Vaccine vs. Control
		% seropositive subject:	68.0% vs. 67.4%
		95%CI:	(65.4;70.5) vs. (63.6;70.9)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **post-Dose 3**	Comparison groups	Vaccine vs. Control
		% seropositive subject:	99.8% vs. 69.1%
		95%CI:	(99.5;100.0) vs. (65.4;72.6)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	Comparison groups	Vaccine vs. Control
		% seropositive subject:	96.8% vs. 72.8%
		95%CI:	(95.7;97.7) vs. (69.2;76.2)
Analysis description	**Secondary analysis (6): Neutralizing antibody level**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1323 (19.3%) and n=660 (19.3%). Data collected from Day 0 to the end of the Active Phase i.e., ., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(1/dil) against each serotype, **baseline**	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 38.3 vs. 42.1 55.3 vs. 62.1 40.1 vs. 40.7 25.3 vs. 26.2

		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (33.8;43.5) vs. (35.0;50.6) (48.7;62.9) vs. (51.7;74.7) (35.6;45.1) vs. (34.5;48.0) (22.9;28.0) vs. (22.6;30.3)
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(1/dil) against each serotype, **post-Dose 3**	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 166.0 vs. 46.6 355.0 vs. 68.5 207.0 vs. 42.5 151.0 vs. 26.0
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (150;183) vs. (38.7;56.1) (327;386) vs. (57.1;82.2) (189;226) vs. (36.2;49.9) (141;162) vs. (22.6;29.8)
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(l/dil) against each serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 90.01 vs. 62.47 150.0 vs. 78.3 118.0 vs. 56.0 70.0 vs. 30.4
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (79.65;102) vs. (51.5;75.6) (135;166) vs. (65.6;93.3) (106;132) vs. (47.3;66.4) (64.1;76.5) vs. (26.2;35.2)
Analysis description	**Secondary analysis (7): Description of baseline neutralizing Abs against Japanese Encephalitis.**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1323 (19.3%) and n=660 (19.3%). Data collected from Day 0 to the end of the Active Phase i.e. up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Baseline neutralizing Abs against Japanese Encephalitis	Comparison groups	Vaccine vs. Control
		% seropositive subject:	53.1% vs. 51.7%
		95%CI:	NA
Analysis description	**Secondary analysis (8): Efficacy against severe VCD during the Active Phase**		
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination.		

Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	6848 (100.0%)	3424 (100.0%)	
	Virologically confirmed **dengue that meets 1997 WHO criteria for DHF** due to any of the four serotypes	8	20	

	Person-years at risk	13857	6917	
	Density incidence (per 100 person-year at risk)	<0.1	0.3	
	95% CI	0.0; 0.1	0.2; 0.4	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Vaccine efficacy	80.0%	
		95%CI	52.7; 92.4	
		P-value	N/A	
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	6848 (100.0%)	3424 (100.0%)	
	Clinically-**severe** virologically-confirmed dengue (**according to the IDMC assessment**) due to any of the four serotypes	12	20	
	Person-years at risk	13853	6917	
	Density incidence (per 100 person-year at risk)	<0.1	0.3	
	95% CI	0.0; 0.2	0.2; 0.4	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Vaccine efficacy	70.0%	
		95%CI	35.7; 86.6	
		P-value	N/A	
Analysis description	**Secondary analysis (9): Occurrence of hospitalized dengue**			
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to 24 months after the third vaccination. Ongoing for data up to 5 years after the third vaccination.			
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	6848 (100.0%)	3424 (100.0%)	

	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Active Phase**	40	61	
	Mean of the number of subjects followed during the years included in the active phase (i.e.,year 1 and year 2)	6830	3416	
	Annual Incidence rate %	0.3	0.9	
	95% CI	0.2; 0.4	0.7; 1.1	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Relative Risk	0.328	
		95%CI	0.21; 0.50	
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	6778 (99.0%)	3387 (98.9%)	
	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Hospital Phase Year 3**	27	13	
	Number of subjects present at the beginning of each period	6778	3387	
	Annual Incidence rate %	0.4	0.4	
	95% CI	0.3; 0.6	0.2; 0.7	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Relative Risk	1.038	
		95%CI	0.52; 2.19	
Analysis description	**Exploratory analysis (1): Efficacy according to age**			
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE); n=6848 (100.0%) and n=3424 (100.0%). Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination			

Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 33.7% 59.5% 74.4%
		95%CI: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 11.7; 50.0 48.9; 68.0 59.2; 84.3
Analysis description	**Exploratory analysis (2): Occurrence of hospitalized dengue according to age**		
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to 24 months after the third vaccination. Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Active Phase**	Comparison groups	Vaccine vs. Control
		Number of cases: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 17 vs. 13 20 vs. 37 3 vs. 11
		Relative Risk: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 0.651 0.271 0.136
		95%CI: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 0.30; 1.46 0.15; 0.48 0.02; 0.51
Effect estimate per comparison	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Hospital Phase, Year 3**	Comparison groups	Vaccine vs. Control
		Number of cases: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 15 vs. 1 10 vs. 8 2 vs. 4
		Relative Risk: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 7.454 0.627 0.249
		95%CI: 2 to 5 years old 6 to 11 years old 12 to 14 years old	 1.15; 313.80 0.22; 1.83 0.02; 1.74
Note:	Data for Hospital Phase are to be updated, only Year 3 is available.		
Analysis description	**Exploratory analysis (3): Efficacy according to baseline serostatus**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE- Subjects included in the immunogenicity subset); n=1335 (19.53%) and n=664 (19.43%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination.		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Number of cases: Dengue immune Dengue non-immune	 18 vs. 34 23 vs. 18
		Efficacy: Dengue immune Dengue non-immune	 74.3% 35.4%

		95%CI: Dengue immune Dengue non-immune	 53.0; 86.0 -27.0; 67.0

Table 14: Summary of Efficacy for trials CYD15

Study Title: Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children and Adolescents Aged 9 to 16 years in Latin America			
Study identifier	**CYD15**		
	Clinical Study Report Up to 24 months post-Injection 3 (Year 1 Hospital Phase)		
Design	Randomized, observer-blind, placebo-controlled, multi-centre, Phase III trial in 5 countries, involving 20,875 subjects. Multi-centre trial conducted at 22 sites across Brazil, Colombia, Honduras, Mexico, and Puerto Rico (approximately 1 to 9 sites in each country).		
	Duration of Active phase: Duration of Hospital phase: Duration of Extension phase:		08 June 2011 - 03 April 2014 (ca. 25 months per subject) 28 May 2013 – 04 March 2015 (ca. 47 months per subject) 2015 – 2018 (ongoing)
Hypothesis	Superiority		
Treatments groups	CYD Dengue Vaccine group		Subjects received 3 vaccinations (0, 6 and 12 months) of CYD Dengue Vaccine with an efficacy follow-up of 13 months after Dose 3 and a follow-up for hospitalized dengue cases of 60 months after Dose 3. **N = 13,917**
	Control Group		Subjects received 3 vaccinations (0, 6 and 12 months) of saline solution (NaCl 0.9%) with an efficacy follow-up of 13 months after Dose 3 and a follow-up for hospitalized dengue cases of 60 months after Dose 3. **N = 6958**
Endpoints and definitions	Primary endpoint	Efficacy	Symptomatic virologically-confirmed dengue cases occurring > 28 days after Dose 3 (during the Active Phase) and defined as: • Acute febrile illness (temperature ⩾ 38°C on at least 2 consecutive days) • Virologically-confirmed by dengue RT-PCR and/or dengue NS1 ELISA Ag test
	Secondary endpoint	Efficacy	Symptomatic virologically-confirmed dengue cases **after the third dose to the end of the Active Phase**: a. due to at least 3 serotypes b. due to each of the 4 serotypes
			Symptomatic virologically-confirmed dengue cases **after at least 1 dose**: a. due to any of the 4 serotypes b. due to at least 3 serotypes c. due to each of the 4 serotypes
			Symptomatic virologically-confirmed dengue cases **after 2 doses**: a. due to any of the 4 serotypes b. due to at least 3 serotypes c. due to each of the 4 serotypes

	Secondary endpoint	Safety	**Occurrence of SAEs**, including serious AESIs, in all subjects throughout the entire study The following serious AESIs were considered: • Serious hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination • Serious viscerotropic disease occurring in all subjects within 30 days after the vaccination • Serious neurotropic disease occurring in all subjects within 30 days after the vaccination • **Serious dengue disease**, as diagnosed by the Investigator, occurring in all subjects at any time during the study
	Secondary endpoint in a subset (N = 2000)	Immunogenicity	Neutralizing Ab level against each of the four parental dengue virus strains of CYD dengue vaccine constructs (and potentially against recently isolated strains) were measured at baseline, after Dose 2, after Dose 3, and 1, 2, 3, 4 and 5 years after Dose 3 (dengue neutralization assay). In addition, baseline neutralizing Abs against YF were described.
	Secondary endpoint in a subset (N = 2000)	Reactogenicity	• unsolicited systemic AEs reported in the 30 minutes after each dose • injection site reactions occurring up to 7 days after each dose • solicited systemic reactions occurring up to 14 days after each dose • unsolicited (spontaneously reported) AEs up to 28 days after each dose. • non-serious AESIs occurring up to 7 days after each dose.
	Other endpoint	Efficacy	Dengue cases during the active phase • Virologically confirmed dengue that meets 1997 WHO criteria for DHF due to any of the four serotypes after each dose. • clinically-severe virologically-confirmed dengue cases due to any of the four serotypes after each dose. • virologically-confirmed hospitalized dengue cases due to each or any of the 4 serotypes. • Symptomatic virologically-confirmed dengue cases due to each or any of the 4 serotypes between each doses
	Other endpoint	Efficacy	Dengue cases during the hospital phase • virologically-confirmed hospitalized dengue cases.
	Other endpoint	Immunogenicity	Serological profile of suspected dengue cases
	Other endpoint	Safety	Dengue viremia • WT dengue strain viremia level in acute samples of virologically confirmed dengue cases.
	Other endpoint	Efficacy Immunogenicity	Relationship Between Neutralizing Ab Level and Vaccine Efficacy • relationship between post-Dose 3 neutralizing Ab level and the subsequent occurrence of symptomatic dengue cases.

	Other endpoint		Medical and Non-Medical Resource Utilization Related to Dengue Disease • level of medical and non-medical resource utilization linked to hospitalized and ambulatory confirmed dengue cases.
Database lock	Database lock for the Active Phase: 22 July 2014 Database lock for the Hospital Phase Year 1: 19 June 2015		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Per Protocol Analysis Set for Efficacy Data collected from 28 days after the third dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.			
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject	12574	6261	
	symptomatic virologically-confirmed dengue cases post-dose 3 due to any of the 4 serotypes	176	221	
	Person-years at risk	11792	5809	
	Density incidence (%)	1.5	3.8	
	95% CI	1.3; 1.7	3.3; 4.3	

Effect estimate per comparison	Primary endpoint	Comparison groups	Vaccine vs. Control
		Vaccine efficacy	60.8
		95%CI	52.0; 68.0
		P-value	The vaccine efficacy is considered as significant if the lower bound of its 95% CI (exact method described by Breslow & Day) is greater than 25%.

Analysis description	**Secondary analysis (1): Efficacy against each serotype after 3 doses**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Modified Full Analysis Set for Efficacy (mFASE); n=13288 (95.5%) and n=6643 (95.6%). Data collected from 28 days after the third dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases post-dose 3, due to each of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 50.3% 42.3% 74.0% 77.7%

		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 29.1; 65.2 14.0; 61.1 61.9; 82.4 60.2; 88.0
Analysis description	**Secondary analysis (2): Efficacy after at least 1 dose**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE); n=13914 (100.0%) and n=6940 (99.9%). Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy:	64.7%
		95%CI:	58.7; 69.8
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to each of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 54.8% 50.2% 74.2% 80.9%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 40.2; 65.9 31.8; 63.6 63.9; 81.7 70.9; 87.7
Analysis description	**Secondary analysis (3): Efficacy after 2 doses**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Other Efficacy Analysis Set (OEAS); n=13506 (97.0%) and n=6765 (97.4%). Data collected from 28 days after the second dose of vaccine to the end of the Active Phase i.e., up to 13 months after the third vaccination.		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after 2 doses, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy:	61.9%
		95%CI:	54.7; 68.0
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after 2 doses, due to each of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Vaccine efficacy: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 50.8% 45.7% 73.3% 81.9%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 33.0; 63.9 23.2; 61.6 61.9; 81.4 69.4; 89.8
Analysis description	**Secondary analysis (4): Seropositivity against each serotype**		

Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1301 (9.3%) and n=643 (9.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against each serotype, **baseline**	Comparison groups	Vaccine vs. Control
		% seropostive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 72.8% vs. 70.5% 76.1% vs. 73.8% 76.5% vs. 73.6% 68.2% vs. 65.0%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (70.3;75.2) vs. (66.8;74.0) (73.6;78.4) vs. (70.2;77.1) (74.1;78.8) vs. (70.0;76.9) (65.6;70.8) vs. (61.2;68.7)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against each serotype, **post-Dose 3**	Comparison groups	Vaccine vs. Control
		% seropostive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 94.9% vs. 74.2% 98.5% vs. 77.2% 98.4% vs. 78.0% 98.1% vs. 68.9%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (93.5;96.0) vs. (70.6;77.6) (97.7;99.1) vs. (73.7;80.4) (97.5;99.0) vs. (74.6;81.1) (97.2;98.8) vs. (65.2;72.5)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against each serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	Comparison groups	Vaccine vs. Control
		% seropostive subject: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 83.9% vs. 75.8% 93.8% vs. 81.3% 94.7% vs. 80.6% 94.3% vs. 72.5%
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (81.8;86.0) vs. (72.2;79.1) (92.3;95.1) vs. (78.0;84.4) (93.3;95.9) vs. (77.3;83.7) (92.8;95.5) vs. (68.8;76.0)
Analysis description	**Secondary analysis (5): Seropositivity against at least one serotype**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1301 (9.3%) and n=643 (9.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **baseline**	Comparison groups	Vaccine vs. Control
		% seropostive subject:	80.6% vs. 77.2%
		95%CI:	(78.4;82.7) vs. (73.8;80.4)
Effect estimate per comparison	Seropositive subjects	Comparison groups	Vaccine vs. Control

	(neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **post-Dose 3**	% seropostive subject:	99.8% vs. 84.1%
		95%CI:	(99.3;100.0) vs. (81.0;86.8)
Effect estimate per comparison	Seropositive subjects (neutralizing Ab titers ⩾ 10 (1/dil)) against at least 1 serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	Comparison groups	Vaccine vs. Control
		% seropostive subject:	99.3% vs. 83.5%
		95%CI:	(98.6;99.7) vs. (80.3;86.4)
Analysis description	**Secondary analysis (6): Neutralizing antibody level**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1301 (9.3%) and n=643 (9.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(l/dil) against each serotype, **baseline**	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 128 vs. 119 138 vs. 115 121 vs. 114 43.6 vs. 39.0
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (112;145) vs. (98.7;142) (123;156) vs. (97.2;136) (108;136) vs. (95.9;136) (39.6;48.0) vs.(33.9;44.7)
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(l/dil) against each serotype, **post-Dose 3**	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 395 vs. 121 574 vs. 129 508 vs. 124 241 vs. 44.3
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (353;441) vs. (101;145) (528;624) vs. (109;152) (465;555) vs. (105;147) (226; 258) vs. (38.6;50.8)
Effect estimate per comparison	Neutralizing Ab titers, PRNT-(l/dil) against each serotype, **2 years after Dose 3** (these data are based on CSR v. 2.0 done in 2015)	Comparison groups	Vaccine vs. Control
		GMTs: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 209 vs. 142 340 vs. 173 302 vs. 170 138 vs. 56.5
		95%CI: Serotype 1 Serotype 2 Serotype 3 Serotype 4	 (185;237) vs. (118;171) (308;375) vs. (146;205) (274;334) vs. (142;203) (128;149) vs. (48.7;65.5)

Analysis description	**Secondary analysis (7): Description of baseline neutralizing Abs against Yellow Fever.**			
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Immunogenicity (FASI); n=1301 (9.3%) and n=643 (9.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.			
Effect estimate per comparison	Baseline neutralizing Abs against Yellow Fever	Comparison groups	Vaccine vs. Control	
		% seropostive subject:	80.1% vs. 79.0%	
		95%CI:	NA	
Analysis description	**Secondary analysis (8): Efficacy against severe virologically confirmed dengue during the Active Phase**			
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination.			
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	13914 (100.0%)	6940 (99.9%)	
	Virologically confirmed **dengue that meets 1997 WHO criteria for DHF d**ue to any of the four serotypes	1	10	
	Person-years at risk	27094	13519	
	Density incidence (per 100 person-year at risk)	<0.1	<0.1	
	95% CI	0.0; 0.0	0.0; 0.1	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Vaccine efficacy	95.0%	
		95%CI	64.9; 99.9	
		P-value	no hypothesis was tested for secondary or other objectives	
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	13914 (100.0%)	6940 (99.9%)	

	Clinically-**severe** virologically-confirmed dengue (**according to the IDMC assessment**) due to any of the four serotypes	1	11	
	Person-years at risk	27094	13519	
	Density incidence (per 100 person-year at risk)	<0.1	<0.1	
	95% CI	0.0; 0.0	0.0; 0.1	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Vaccine efficacy	95.5%	
		95%CI	68.8; 99.9	
		P-value	no hypothesis was tested for secondary or other objectives	
Analysis description	**Secondary analysis (9): Occurrence of hospitalized dengue**			
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to 24 months after the third vaccination. Ongoing for data up to 5 years after the third vaccination.			
Descriptive statistics and estimate variability	Treatment group	CYD Dengue Vaccine Group	Control	
	Number of subject and % of the enrolled subjects	13915 (100.0%)	6939 (99.9%)	
	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Active Phase**	17	43	
	Mean of the number of subjects followed during the years of the active phase	13719	6844	
	Annual Incidence rate %	<0.1	0.3	
	95% CI	0.0; 0.1	0.2; 0.4	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Relative Risk	0.197	
		95%CI	0.11; 0.35	
Descriptive statistics and estimate	Treatment group	CYD Dengue Vaccine Group	Control	

variability	Number of subject and % of the enrolled subjects	13915 (100.0%)	6939 (99.9%)	
	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Hospital Phase Year 3**	16	15	
	Number of subjects present at the beginning of each period	13268	6630	
	Annual Incidence rate %	0.1	0.2	
	95% CI	0.1; 0.2	0.1; 0.4	
Effect estimate per comparison	Secondary endpoint	Comparison groups	Vaccine vs. Control	
		Relative Risk	0.533	
		95%CI	0.25; 1.16	
Note:	Data for Hospital Phase are to be updated, only Year 3 is currently available.			
Analysis description	**Exploratory analysis (1): Efficacy according to age**			
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE); n=13914 (100.0%) and n=6940 (99.9%). Data collected from Day 0 (first dose) to the end of the Active Phase i.e., up to 13 months after the third vaccination			
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control	
		Vaccine efficacy: 9 to 11 years old 12 to 16 years old	 61.7% 67.6%	
		95%CI: 9 to 11 years old 12 to 16 years old	 52.3; 69.3 59.3; 74.3	
Analysis description	**Exploratory analysis (2): Occurrence of hospitalized dengue according to age**			
Analysis population and time point description	Full Analysis Set for Efficacy (FASE) Data collected from Day 0 (first dose) to 24 months after the third vaccination. Ongoing for data up to 5 years after the third vaccination.			
Effect estimate per comparison	Virologically confirmed hospitalized dengue due to any of the four serotypes, **Active Phase**	Comparison groups	Vaccine vs. Control	
		Number of cases: 9 to 11 years old 12 to 16 years old	 8 vs. 22 9 vs. 21	
		Relative Risk: 9 to 11 years old 12 to 16 years old	 0.181 0.214	
		95%CI: 9 to 11 years old 12 to 16 years old	 0.07; 0.42 0.09; 0.49	
Effect estimate per comparison	Virologically confirmed	Comparison groups	Vaccine vs. Control	

	hospitalized dengue due to any of the four serotypes, **Hospital Phase, Year 3**	Number of cases: 9 to 11 years old 12 to 16 years old	 10 vs. 9 6 vs. 6
		Relative Risk: 9 to 11 years old 12 to 16 years old	 0.554 0.501
		95%CI: 9 to 11 years old 12 to 16 years old	 0.20; 1.54 0.13; 1.87
Note:	Data for Hospital Phase are to be updated, only Year 3 is available		
Analysis description	**Exploratory analysis (3): Efficacy according to baseline serostatus**		
Analysis population and time point description	Population; n (number of subjects) and % of the enrolled subjects, respectively in the Vaccine and the Control Groups: Full Analysis Set for Efficacy (FASE – subjects in the immunogenicity dataset); n=1301 (9.3%) and n=643 (9.3%). Data collected from Day 0 to the end of the Active Phase i.e., up to 13 months after the third vaccination; Ongoing for data up to 5 years after the third vaccination.		
Effect estimate per comparison	Symptomatic virologically-confirmed dengue cases after at least 1 dose, due to any of the 4 serotypes	Comparison groups	Vaccine vs. Control
		Number of cases: Dengue immune Dengue non-immune	 8 vs. 23 9 vs. 9
		Efficacy: Dengue immune Dengue non-immune	 83.7% 43.2%
		95%CI: Dengue immune Dengue non-immune	 62.0; 94.0 -62.0; 80.0

2.5.3. Analysis performed across trials (pooled analyses and meta-analysis)

The three efficacy studies CYD14, CYD15 and CYD23 were each statistically powered to demonstrate efficacy. This complies with the FDA guideline that recommends securing conclusions based on at least 2 persuasive studies. In addition to the results presented above for each individual study, a meta-analysis was performed on the efficacy studies to summarize the overall efficacy of the CYD dengue vaccine evaluated in different settings.

This Integrated Efficacy Analysis (IEA) was performed for the following purposes:

- To improve the precision of the estimates for specific endpoints and analyses:
  1. VE for clinically severe VCD cases
  2. VE for VCD cases that meets WHO criteria for dengue haemorrhagic fever (DHF)
  3. VE for hospitalized VCD cases
  4. VE by serotype
- To assess the impact of some covariates (age, gender, dengue status at baseline, etc…) on the VE; especially when the covariate is assessed in a subpopulation (dengue immune status assessed in the immunogenicity subset).

Comparison and Analyses of Efficacy Results Across Studies

The integrated results of efficacy are presented first by the individual VE estimates for the 2 pivotal efficacy studies CYD14 and CYD15, supporting the vaccine efficacy claim. Then, the integrated VE estimate from the meta-analysis on CYD14 + CYD15 is provided as supportive data to provide an overall estimate with more precision, which is important for exploratory endpoints. Potential heterogeneity between the 2 studies, i.e. p value < 10%, is discussed on a case-by-case basis, considering statistical aspects as well as clinical significance.

Finally, the individual VE estimate for the proof-of-concept study CYD23 and the sensitivity analysis of the integrated estimate from a meta-analysis on CYD14 + CYD15 + CYD23 are provided as supportive data.

The following five points are evaluated:

1. The study populations for efficacy analysis in terms of demographic characteristics;
2. Disposition of subjects and baseline FV/dengue immune status;
3. Comparison of Efficacy Results of all Studies: main, secondary and other objectives of VE;
4. The impact of covariates;
5. A focus on the subset of subjects from the claimed population in the indication where VE after 3 injections of the CYD dengue vaccine administered 6 months apart was collected, i.e. in subjects from 9 to 16 years as per efficacy studies design.

Demographic Characteristics

The demographic characteristics of subjects in all analysis sets in the efficacy studies are presented in Table 15:

Table 15: Demographics at baseline, all analysis sets

		CYD Dengue Vaccine (N=23440)					Control (N=11706)				
Analysis Set	Studies	Male n (%)	Mean Age (years)	Children 2-5 years n (%)	Children 6-11 years n (%)	Adolescents 12-16 years n (%)	Male n (%)	Mean Age (years)	Children 2-5 years n (%)	Children 6-11 years n (%)	Adolescents 12-16 years n (%)
PPSE	CYD14	3252 (48.5)	8.8	1615 (24.1)	3567 (53.2)	1527 (22.8)	1623 (48.4)	8.8	795 (23.7)	1793 (53.5)	762 (22.7)
	CYD15	6253 (49.7)	12.4	0 (0.0)	5770 (45.9)	6803 (54.1)	3105 (49.6)	12.4	0 (0.0)	2860 (45.7)	3401 (54.3)
	CYD14+CYD15	9505 (49.3)	11.2	1615 (8.4)	9338 (48.4)	8330 (43.2)	4728 (49.2)	11.2	795 (8.3)	4653 (48.4)	4163 (43.3)
	CYD23	1187 (48.4)	8.2	443 (18.1)	2009 (81.9)	0 (0.0)	583 (47.7)	8.2	209 (17.1)	1012 (82.9)	0 (0.0)
	CYD14+CYD15+CYD23	10692 (49.2)	10.8	2058 (9.5)	11346 (52.2)	8330 (38.3)	5311 (49.0)	10.8	1004 (9.3)	5665 (52.3)	4163 (38.4)
mFASE	CYD14	3287 (48.5)	8.8	1632 (24.1)	3599 (53.1)	1541 (22.8)	1637 (48.4)	8.8	810 (24.0)	1803 (53.4)	766 (22.7)
	CYD15	6598 (49.7)	12.4	0 (0.0)	6084 (45.8)	7204 (54.2)	3281 (49.4)	12.4	0 (0.0)	3031 (45.6)	3612 (54.4)
	CYD14+CYD15	9885 (49.3)	11.2	1632 (8.1)	9683 (48.3)	8745 (43.6)	4918 (49.1)	11.2	810 (8.1)	4834 (48.2)	4378 (43.7)
	CYD23	1236 (48.3)	8.2	463 (18.1)	2094 (81.9)	0 (0.0)	610 (47.6)	8.2	219 (17.1)	1063 (82.9)	0 (0.0)
	CYD14+CYD15+CYD23	11121 (49.2)	10.9	2095 (9.3)	11777 (52.1)	8745 (38.7)	5528 (48.9)	10.9	1029 (9.1)	5897 (52.2)	4378 (38.7)
FASE	CYD14	3324 (48.5)	8.8	1655 (24.2)	3638 (53.1)	1555 (22.7)	1657 (48.4)	8.8	826 (24.1)	1824 (53.3)	774 (22.6)
	CYD15	6876 (49.4)	12.5	0 (0.0)	6305 (45.3)	7609 (54.7)	3412 (49.2)	12.5	0 (0.0)	3146 (45.3)	3794 (54.7)
	CYD14+CYD15	10200 (49.1)	11.3	1655 (8.0)	9943 (47.9)	9164 (44.1)	5069 (48.9)	11.3	826 (8.0)	4970 (48.0)	4568 (44.1)
	CYD23	1290 (48.4)	8.2	482 (18.1)	2184 (81.9)	0 (0.0)	635 (47.7)	8.2	230 (17.3)	1101 (82.7)	0 (0.0)
	CYD14+CYD15+CYD23	11490 (49.0)	10.9	2137 (9.1)	12127 (51.8)	9164 (39.1)	5704 (48.8)	10.9	1056 (9.0)	6071 (51.9)	4568 (39.1)
FASI	CYD14	652 (49.3)	8.6	455 (34.4)	468 (35.4)	400 (30.2)	310 (47.0)	8.6	223 (33.8)	238 (36.1)	199 (30.2)
	CYD15	631 (48.5)	12.3	0 (0.0)	643 (49.4)	658 (50.6)	339 (52.7)	12.4	0 (0.0)	324 (50.4)	319 (49.6)
	CYD14+CYD15	1283 (48.9)	10.4	455 (17.3)	1111 (42.3)	1058 (40.3)	649 (49.8)	10.5	223 (17.1)	562 (43.1)	518 (39.8)
	CYD23	84 (42.6)	8.3	26 (13.2)	171 (86.8)	0 (0.0)	46 (46.5)	8.1	14 (14.1)	85 (85.9)	0 (0.0)
	CYD14+CYD15+CYD23	1367 (48.5)	10.3	481 (17.1)	1282 (45.4)	1058 (37.5)	695 (49.6)	10.3	237 (16.9)	647 (46.1)	518 (36.9)

n: number of subjects fulfilling the item listed in the considered population

The demographic data of subjects from the efficacy studies were comparable across the various analysis sets and between the Dengue Group and the Control Group. In CYD14, the age distribution in the PPSE, mFASE and FASE showed a higher proportion of children aged 6 to 11 years (approximately 53%) compared with children aged 2 to 5 years and adolescents aged 12 to 14 years (approximately 24% and 23%, respectively). In CYD15 the age distribution in the PPSE, mFASE and FASE showed a higher proportion of adolescent (approx. 55%) compared with children 6 to 11 years (approx. 45%). In the FASI, age distribution was quite homogeneous for both studies.

The disposition of subjects in the various analysis sets used for efficacy in CYD14, CYD15 and CYD23 are presented in Table 16 in randomized subjects.

Table 16: Efficacy analysis sets by randomised group- randomised subjects

	CYD Dengue Vaccine					Control				
Studies	N	PPSE n (%)	FASE n (%)	mFASE n (%)	FASI n (%)	N	PPSE n (%)	FASE n (%)	mFASE n (%)	FASI n (%)
CYD14	6851	6709 (97.9)	6848 (100.0)	6772 (98.8)	1323 (19.3)	3424	3350 (97.8)	3424 (100.0)	3379 (98.7)	660 (19.3)
CYD15	13920	12573 (90.3)	13914 (100.0)	13288 (95.5)	1301 (9.3)	6949	6261 (90.1)	6940 (99.9)	6643 (95.6)	643 (9.3)
CYD14+CYD15	20771	19282 (92.8)	20762 (100.0)	20060 (96.6)	2624 (12.6)	10373	9611 (92.7)	10364 (99.9)	10022 (96.6)	1303 (12.6)
CYD23	2669	2452 (91.9)	2666 (99.9)	2557 (95.8)	197 (7.4)	1333	1221 (91.6)	1331 (99.8)	1282 (96.2)	99 (7.4)
CYD14+CYD15+CYD23	23440	21734 (92.7)	23428 (99.9)	22617 (96.5)	2821 (12.0)	11706	10832 (92.5)	11695 (99.9)	11304 (96.6)	1402 (12.0)

n: number of subjects fulfilling the item listed

In both pivotal studies, almost all randomized subjects received at least one injection of either the CYD dengue vaccine or the control. In the Dengue Group, the FASE represented 6848 subjects from CYD14 and 13,914 subjects from CYD15.

The PPSE included 6709 subjects from CYD14 and 12,573 subjects from CYD15 in the Dengue Group, representing a 97.9% and 90.3% compliance to the protocol, respectively. The mFASE was near 99.0% for CYD14 and near 95.5% for CYD15.

The different populations highlight a high compliance to the vaccination schedule, with more than 95% of subjects receiving 3 injections of either the CYD dengue vaccine or the control. Again, compliance in the FASI was high, with 1323/1336 subjects included in CYD14 and 1301/1334 subjects included in CYD15.

Overall compliance was good in all efficacy studies, and all analysis sets remained within the 2:1 ratio for receipt of the CYD dengue vaccine or placebo. The great majority of subjects fulfilled the Per-Protocol requirements. Therefore, in these two studies, the PPSE and mFASE populations are almost equivalent in terms of interpretation of results.

The dengue and other FV immune status at baseline of subjects from the immunogenicity subset in CYD14, CYD15 and CYD23 are presented in Table 17. In AP endemic regions, i.e. CYD14 and CYD23, JE was tested at baseline; in LatAm endemic regions, i.e. CYD15, YF was tested at baseline.

Table 17: Baseline Flavivirus immune status – Subset - FASI

	CYD Dengue Vaccine					Control Group				
Studies	N	Dengue Non immune n/M (%)	Dengue immune n/M (%)	JE/YF* Immune n/M (%)	Flavivirus Immune n/M (%)	N	Dengue Non immune n/M (%)	Dengue immune n/M (%)	JE/YF* Immune n/M (%)	Flavivirus Immune n/M (%)
CYD14	1323	419/1315 (31.9)	896/1315 (68.1)	702/1319 (53.2)	1042/1317 (79.1)	660	212/656 (32.3)	444/656 (67.7)	341/658 (51.8)	509/657 (77.5)
CYD15	1301	251/1299 (19.3)	1048/1299 (80.7)	1042/1295 (80.5)	1128/1299 (86.8)	643	145/640 (22.7)	495/640 (77.3)	508/638 (79.6)	543/639 (85.0)
CYD14+CYD15	2624	670/2614 (25.6)	1944/2614 (74.4)	1744/2614 (66.7)	2170/2616 (83.0)	1303	357/1296 (27.5)	939/1296 (72.5)	849/1296 (65.5)	1052/1296 (81.2)
CYD23	197	59/197 (29.9)	138/197 (70.1)	157/197 (79.7)	179/197 (90.9)	99	31/99 (31.3)	68/99 (68.7)	77/98 (78.6)	91/99 (91.9)
CYD14+CYD15+CYD23	2821	729/2811 (25.9)	2082/2811 (74.1)	1901/2811 (67.6)	2349/2813 (83.5)	1402	388/1395 (27.8)	1007/1395 (72.2)	926/1394 (66.4)	1143/1395 (81.9)

JE: Japanese Encephalitis. YF: Yellow Fever. FV: Flavivirus.
* JE assessed in endemic Asia Pacific studies, YF assessed in endemic Latin America studies
Dengue immune subjects at baseline are defined as those subjects with titers ≥ LLOQ (i.e. 10) (1/dil) against at least one dengue serotype at baseline. Dengue non-immune subjects at baseline are defined as subjects with titers at baseline for the 4 serotypes < LLOQ.
Baseline flavivirus immune subjects are defined as those subjects with titers ≥ LLOQ (1/dil) against at least one dengue serotype, or JE, or YF at baseline
Subjects with undetermined baseline status (no titer ≥ LLOQ and at least one missing titer) are excluded
n: number of subjects fulfilling the item listed. For Serotype 1 to 4, number of subjects with titers ≥ LLOQ (i.e. 10) (1/dil) against this dengue serotype at baseline
M: number of subjects with available data
Immunoassays are Dengue PRNT, PRNT for JE, and PRNT for YF

In CYD14 Dengue Group, 68.1% of subjects were dengue immune at baseline and 53.2% of subjects were JE immune at baseline. In CYD15 Dengue Group, 80.7% of subjects were dengue immune at baseline and 80.5% of subjects were YF immune at baseline. This result reflects the older age group recruited in CYD15 and the different regional epidemiology in LatAm versus Asia. The proportions of FV

and dengue immune subjects at baseline were high for all efficacy studies. In each of the trials, subjects from the Dengue Group and from the Control Group had comparable baseline immune status.

Dengue Cases in the Control Group

Dengue cases reported in the Control Group are presented to reflect the epidemiology in the population over the course of the study in the absence of the CYD dengue vaccine. The density incidence, the number of VCD cases and the serotype distribution in the Control Group in the 3 efficacy studies, during the PD3 period (mFASE) and during the whole active phase (FASE) are described below. In addition, the distribution of each of the 4 serotypes for all VCD cases occurring in the Control Group is presented both during the PD3 and the whole Active Phase periods in Table 18 and Figure 7.

Table 18: Density incidence, number of VCD cases and serotype distribution in the Control Group – various analysis sets

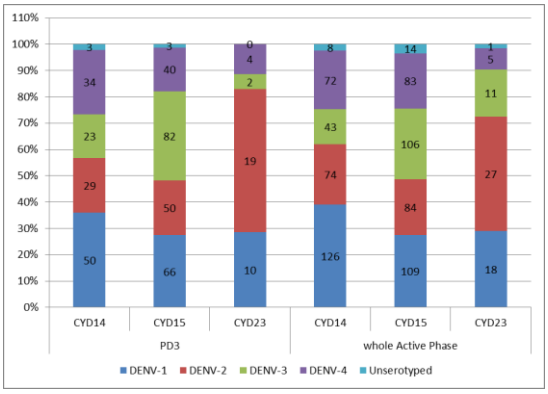
	CYD14		CYD15		CYD23	
Period and population	**PD3 (mFASE)**	**whole Active Phase (FASE)**	**PD3 (mFASE)**	**whole Active Phase (FASE)**	**PD3 (mFASE)**	**whole Active Phase (FASE)**
Density incidence* (95% CI) (any serotype) in Controls	4.1 (3.5 ; 4.9)	4.7 (4.2; 5.2)	3.8 (3.4 ; 4.3)	2.9 (2.6 ; 3.2)	2.6 (1.8; 3.6)	2.2 (1.7; 2.8)
Dengue episodes (any serotype) in Controls (n)	135	319	236	388	35	62
Serotype distribution in Controls (Subjects with at least one VCD cases)						
Serotype 1	50	126	66	109	10	18
Serotype 2	29	74	50	84	19	27
Serotype 3	23	43	82	106	2	11
Serotype 4	34	72	40	83	4	5
Unserotyped	3	8	3	14	0	1

* Density incidence: cases per 100 person-years at risk

During the conduct of the Active Phase, the dengue incidence in the control group was higher than incidence rates reported by the passive surveillance systems in the municipalities where the studies were conducted and which were used for the sample size calculation:

- In CYD14 the incidence in the control group was 4.7% during the whole Active Phase versus 1.3% used to estimate the sample size of the study.
- In CYD15 the incidence in the control group was 2.9% during the whole Active Phase versus 0.64% used to estimate the sample size of the study.

Figure 7: Distribution of each of the 4 serotypes in all VCD cases in the Control Group – mFASE for PD3 and FASE for whole Active Phase

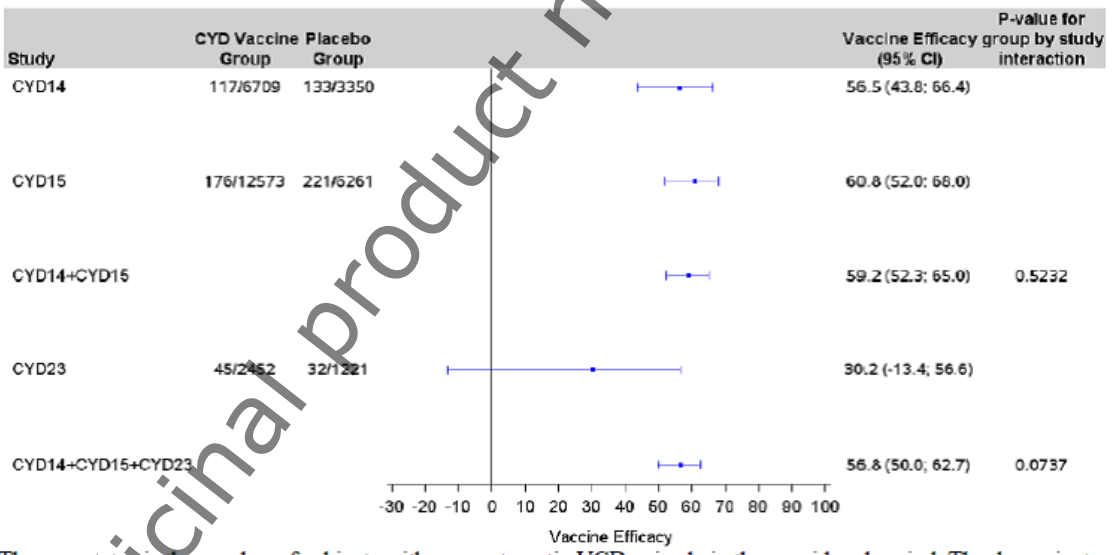


Overall, the efficacy studies cover various epidemiological settings in different endemic regions, with different density incidences. In the 2 pivotal efficacy studies, each of the 4 serotypes is represented in sufficient proportions, making it possible to assess VE against any and each of these 4 serotypes.

Primary Objective: VE against symptomatic VCD by any serotype in the PD3 period

VE against VCD cases PD3 due to any of the 4 serotypes in the PPSE is presented in the next Figure 8. This analysis is based on the number of cases, defined as the number of subjects with at least one symptomatic VCD episode from 28 days post-injection 3 to the end of the Active Phase.

Figure 8: Forrest plot for VE against symptomatic VCD cases PD3 due to any of the 4 serotypes - PPSE



The numerator is the number of subjects with a symptomatic VCD episode in the considered period. The denominator is the number of subjects. VE of a study is calculated using density incidence: cases per 100 person-years at risk. Integrated VE and CIs are calculated using Cox regression model.

The VE against VCD cases due to any serotype was demonstrated in the 2 pivotal efficacy studies in the PD3 period:

- In CYD14, the VE estimates against VCD cases due to any serotype in the PD3 period was 56.5% (95% CI: 43.8; 66.4), with the lower bound of the 95% CI above 25%, thereby reaching the primary objective.

- In CYD15, the VE estimates against VCD cases due to any serotype in the PD3 period was 60.8% (95% CI: 52.0; 68.0), with the lower bound of the 95% CI above 25%, thereby reaching the primary objective.

The 2 pivotal efficacy studies had consistent results with a heterogeneity test of 0.5235, i.e. p-value ≥10%.

The pooled analysis of CYD14 + CYD15 VE estimate against VCD cases due to any serotype was 59.2%, with a 95% CI of 52.3-65.0. Results were confirmed in the mFASE for individual studies and the pooled meta-analysis.

In CYD23, VE was 30,2%, but the 95% CI includes 0, so VE could not be demonstrated in this trial. The CYD23 result was primarily driven by the data that approximately 60% of the VCD cases in which the serotype was known was due to serotype 2, against which VE was not demonstrated. This result probably highlights the limitation to conduct the study in only one centre in a single area of Thailand, where there is a risk that a single lineage of a single serotype circulates during the efficacy assessment.

When combining data from CYD23 with CYD14 + CYD15, the heterogeneity test of the pooled analysis of CYD14+CYD15+CYD23 showed a p-value < 10%. Despite the results of CYD23, the pool provides similar estimates as the impact of CYD23 as compared with CYD14 and CYD15 is low in terms of number of cases.

Results from the mFASE including only subjects >9YOA as per indication are provided below:

Table 19: VE against VCD cases PD3 due to any of the 4 serotypes in subjects 9 to 16 years

	CYD14		**CYD15**		**CYD23**		**Pooled CYD14+CYD15**		**Pooled ** CYD14+CYD15+ CYD23**	
	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group
Cases / person-years	34/3199	55/1585	185/ 12458	236/6157	6/1033	10/514	219/15657	291/7742	225/16690	301/8256
VE %* (95%CI)	69.4 (52.2; 80.6)		61.3 (52.8; 68.2)		70.1 (9.3; 91.1)		62.8 (55.7; 68.8)		63.0 (56.1; 68.9)	

N: number of subjects per study. * The efficacy of Dengvaxia is considered as significant if the lower bound of the 95% CI is greater than 25% (CYD14 and CYD15) or greater than 0% (CYD23). **Pooled results of CYD14, 15 and 23 need to be interpreted cautiously because of differences in the Dengue confirmatory test and acute febrile illness definition between CYD14/15 and CYD23.

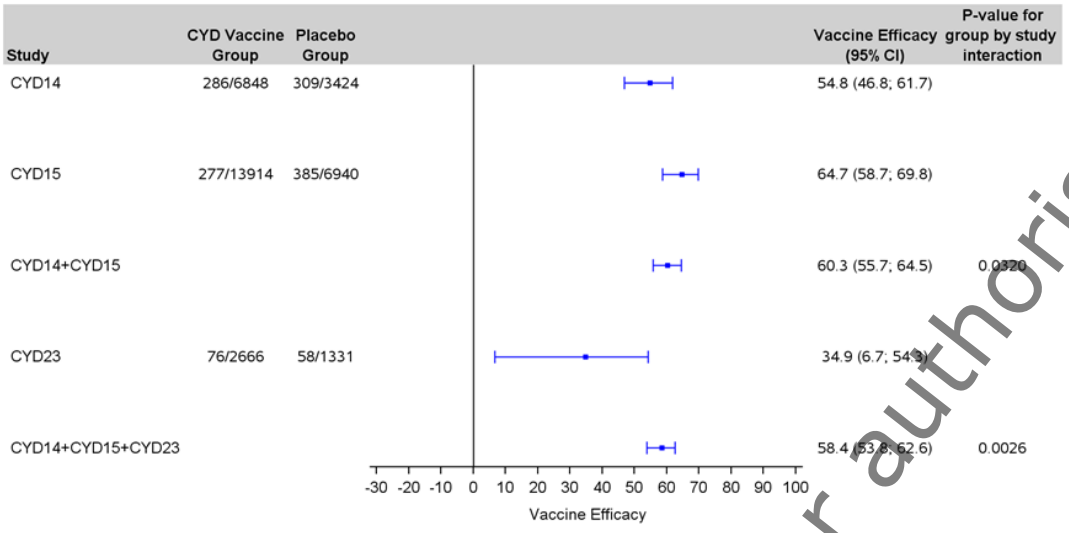
Secondary Objectives

All secondary objectives are presented during the whole Active Phase period (FASE) and confirmed during the PD3 period for serotype-specific analysis.

1. VE due to any serotype

VE against VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASE population is presented in Figure 9. This analysis is based on the number of cases, defined as the number of subjects with at least one symptomatic VCD episode after at least 1 injection till the end of the Active Phase (25 months) due to any of the 4 serotypes.

Figure 9: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to any of the 4 serotypes – FASE



VE against VCD cases due to any serotype in a population aged 2 to 16 years living in dengue-endemic regions was demonstrated in the 2 pivotal efficacy studies for the whole Active Phase period (25 months PD1) and was consistent with those observed during the PD3 period (12 months PD3) (PPSE). In this population (FASE), VE in trial CYD23 was lower than in trials CYD14 and CYD15, but it was higher than 0, since the lower bound of the 95% CI was above 0.

2. VE Due to Each Serotype

VE against VCD cases during the whole Active Phase in the FASE is presented for serotypes 1 to 4 in the next Figures. In these figures, the numerator is the number of subjects with a symptomatic VCD episode in the considered period and the denominator is the number of subjects; VE of a study is calculated using density incidence: cases per 100 person-years at risk; integrated VE and CIs are calculated using Cox regression model.

Figure 10: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to serotype 1 - FASE

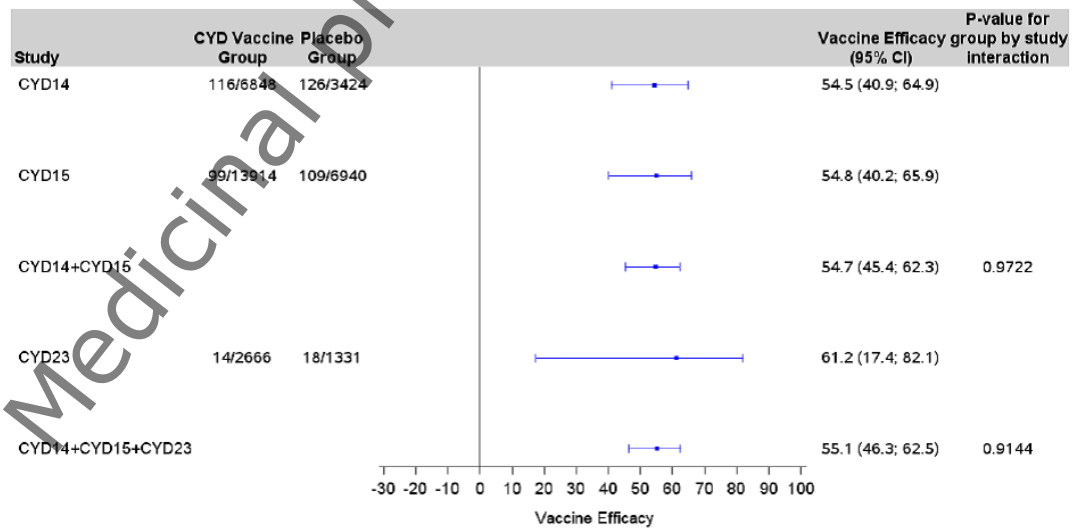


Figure 11: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to serotype 2 – FASE

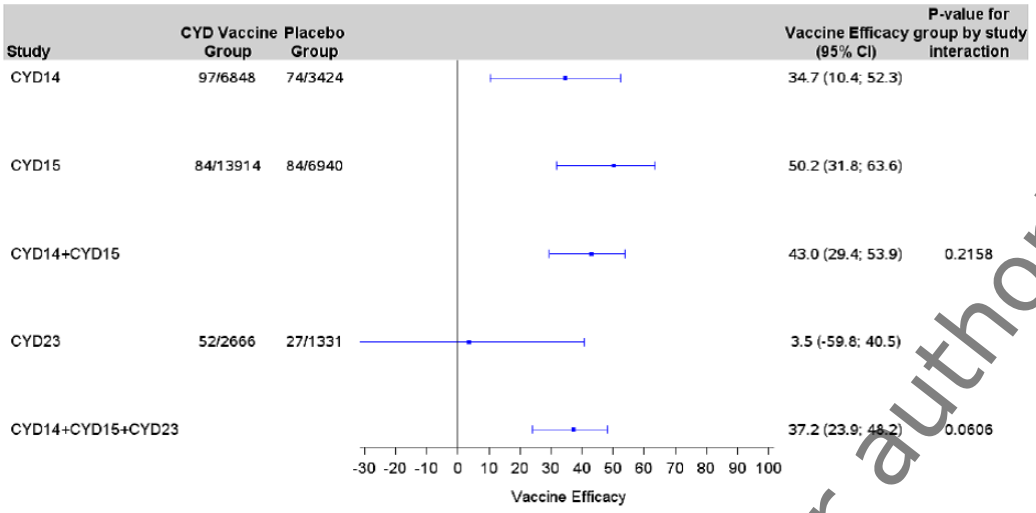


Figure 12: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to serotype 3 – FASE

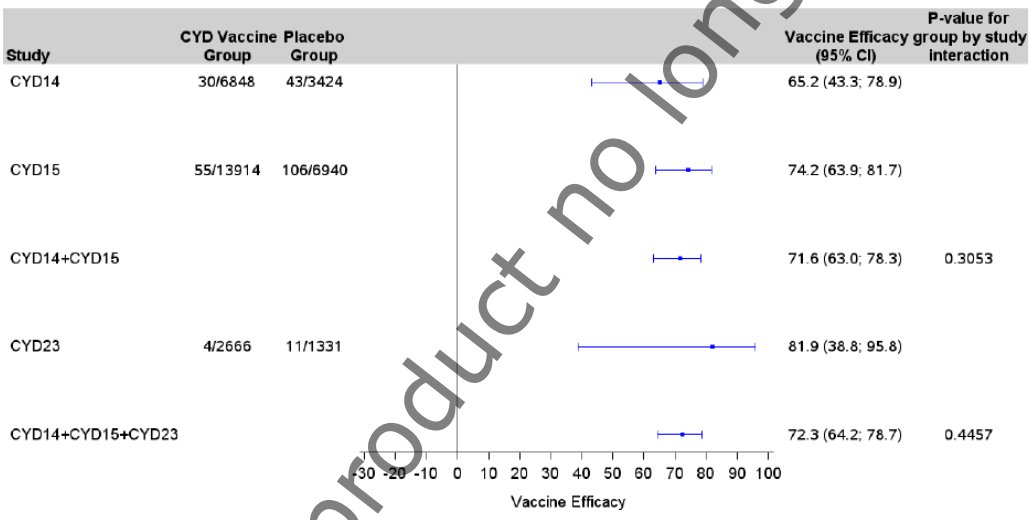
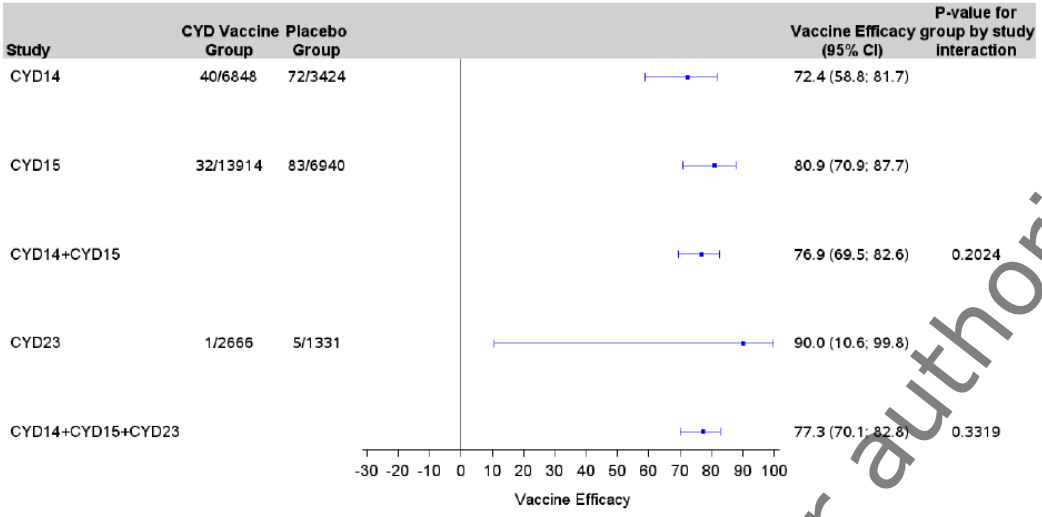


Figure 13: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to serotype 4 – FASE



VE against VCD cases was demonstrated in the 2 pivotal efficacy studies for each of the 4 serotypes during the whole Active Phase period:

- In CYD14, the number of VCD cases ranged from 73 for serotype 3 to 242 for serotype 1. During the whole Active Phase period, VE estimates for each serotype ranged from 34.7% (95% CI: 10.4; 52.3) for serotype 2 to 72.4% (95% CI: 58.8; 81.7) for serotype 4, always with the lower bounds of the 95% CIs above 0.
- In CYD15, the number of VCD cases ranged from 115 for serotype 4 to 208 for serotype 1. During the whole Active Phase period, VE estimates for each serotype ranged from 50.2% (95% CI: 31.8; 63.6) for serotype 2 to 80.9% (95% CI: 70.9; 87.7) for serotype 4, always with the lower bounds of the 95% CIs above 0.

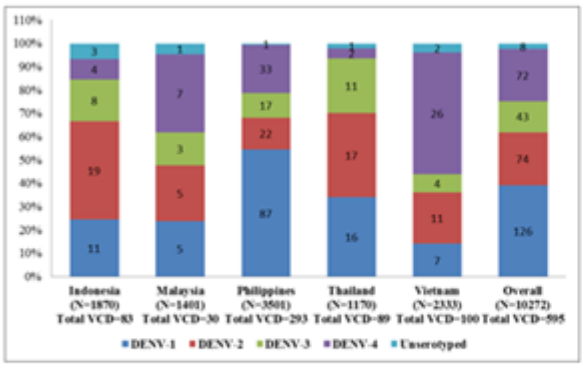
Although the VE estimate for serotype 2 was lower in CYD14 as compared with CYD15, the 2 pivotal efficacy studies had consistent results for each serotype based on heterogeneity tests, with a lower VE for serotypes 1 and 2 as compared with serotypes 3 and 4. Multinomial models confirmed that VE for serotypes 1 and 2 were similar, that VE for serotypes 3 and 4 were similar and that VE for serotypes 1 and 2 were lower than to serotypes 3 and 4. Importantly, the 95% CI of the VE estimates for serotypes 3 and 4 do not overlap with those determined for serotypes 1 and 2.

The pooled analysis of CYD14 + CYD15 confirmed the individual study results with VE estimates ranging from 43.0% (95% CI: 29.4; 53.9) for serotype 2 to 76.9% (95% CI: 69.5; 82.6) for serotype 4.

Overall, the VE estimates against each serotype during the whole Active Phase are consistent with the PD3 VE estimates for serotypes 1, 3 and 4. VE PD3 for serotype 2 in CYD14 was measurable but inconclusive (35.0%, 95% CI: -9.2; 61.0). A possible explanation to this inconsistency is the moderate VE estimates against this serotype combined with a lower precision due to a limited number of VCD cases compared to other serotypes.

Implications of the variability of the VE estimates by serotype

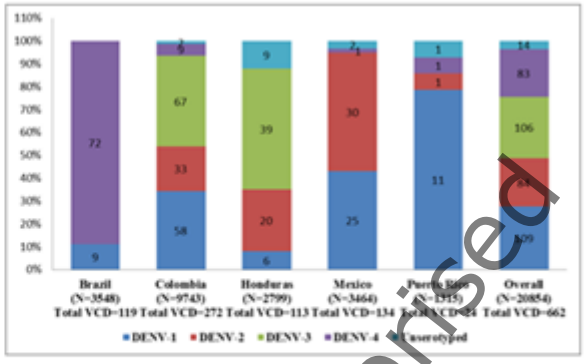
Considering the variability of VE to each serotype, the circulating serotypes in specific settings are expected to impact the overall VE estimate for this location. The distribution of serotypes varied across countries. The distribution of each serotype for all VCD cases occurring during the whole Active Phase, in the Control Group, is presented below:



Total VCD includes all symptomatic VCD episodes from both Dengue Group and Control Group

Source: Modified from 5.3.5.1 CYD14 Report, Appendix 15, Table 24

Figure 14: Distribution of VCD cases per serotype and country in the Control Group during the whole Active Phase in CYD14-FASE



Total VCD includes all symptomatic VCD episodes from both Dengue Group and Control Group

Source: Modified from 5.3.5.1 CYD15 Report, Appendix 15, Table 24

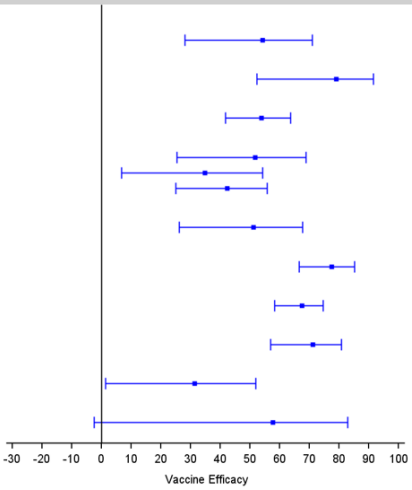
Figure 15: Distribution of VCD cases per serotype and country in the Control Group during the whole active phase in CYP15-FASE

Figure 14and Figure 15 highlight differences of serotype distribution at the country level across studies and across countries within the same studies. Each of the 4 serotypes was represented in each Asian country, whereas some serotypes were not represented in some LatAm countries, i.e. serotype 3 was not present in Brazil whereas serotypes 1 and 2 were the main represented serotypes in Mexico, and serotype 1 was the main serotype represented in Puerto Rico. These differences in circulating serotype during the study drive the variability observed in the VE estimates in each country.

VE against VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASE is presented by country in Figure 16. This analysis is based on the number of cases, defined as the number of subjects with at least one symptomatic VCD episode from at least 1 injection to the end of the Active Phase. The numerator is the number of subjects with a symptomatic VCD episode in the considered period. The denominator is the number of subjects. VE of a study is calculated using density incidence: cases per 100 person-years at risk. Integrated VE and CIs are calculated using Cox regression model.

Figure 16: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to any of the 4 serotypes according to country by study– FASE

Study	CYD Vaccine Group	Placebo Group	Vaccine Efficacy (95% CI)	P-value for group by study interaction
Indonesia				
CYD14	40/1246	43/624	54.3 (28.0; 71.0)	
Malaysia				
CYD14	9/937	21/464	79.0 (52.3; 91.5)	
Philippines				
CYD14	143/2334	150/1166	53.9 (41.7; 63.6)	
Thailand				
CYD14	44/778	45/392	51.8 (25.3; 68.9)	
CYD23	76/2666	58/1331	34.9 (6.7; 54.3)	
CYD14+CYD23			42.4 (25.0; 55.7)	0.2662
Vietnam				
CYD14	50/1553	50/778	51.1 (26.1; 67.6)	
Brazil				
CYD15	38/2369	81/1175	77.5 (66.5; 85.1)	
Colombia				
CYD15	108/6495	164/3245	67.5 (58.3; 74.7)	
Honduras				
CYD15	42/1865	71/931	71.1 (57.0; 80.7)	
Mexico				
CYD15	78/2310	56/1149	31.3 (1.3; 51.9)	
Puerto Rico				
CYD15	11/875	13/440	57.6 (-2.5; 82.8)	



VE estimates against VCD cases due to any serotypes in each country showed some variability:

- In CYD14, the VE during the whole Active Phase ranged from 51.1% (95% CI: 26.1; 67.6) in Viet Nam to 79.0% (95% CI: 52.3; 91.5) in Malaysia;
- In CYD15, the VE during the whole Active Phase ranged from 31.3% (95% CI: 1.3; 51.9) in Mexico to 77.5% (95% CI: 66.5; 85.1) in Brazil.

In CYD15, the VE estimates by country were more variable than in CYD14. This might be explained by the following. Each of the 4 serotypes was represented in each Asian country, whereas some serotypes were not represented in some LatAm countries. In the particular case of Mexico, circulation of mainly serotypes 1 and 2, against which the efficacy of the vaccine appears to be lower as compared with the other 2 serotypes, combined with lower baseline rates of dengue seropositivity, which is a known covariate of VE, may have been the main factors that led to lower overall VE in the country. Whereas in Brazil, higher baseline rates of dengue seropositivity combined with circulation of mainly serotype 4, against which the efficacy of the vaccine appears to be higher as compared with serotypes 1 and 2, may have been the main factors that led to higher overall VE in the country.

3. VE against symptomatic VCD after different doses (Secondary efficacy endpoint)

VE after different doses, i.e. after at least one dose (FASE), after 2 doses (OEAS) after 3 doses (mFASE) was assessed as secondary objective for any and each serotype. Pooled analyses were presented for post-dose 1 (FASE; CYD14+CYD15: 60.3, 95% CI 55.7; 64.5) and post-dose 3 (mFASE, CYD14+CYD15: 59.5, 95% CI 52.9; 65.2) VE estimates, but not for post-dose 2 (OEAS). The pooled analyses shows that VE remains largely stable from the first dose, and overall, no increase in VE estimate is observed after the third dose compared to post-dose 1.

Serotype-specific differences are observed: VE estimate against serotype 1 and 2 tends to decrease with increasing doses, whereas VE estimate against serotype 3 and 4 tends to increase or remain stable, resp., with increasing doses. This trend is consistent through studies CYD14 and CYD15.

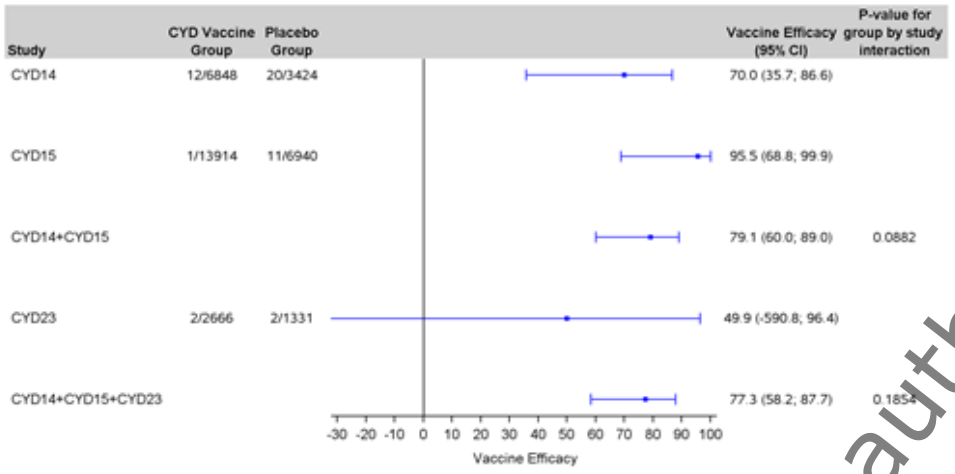
Other Efficacy Objectives

The efficacy studies were not designed to demonstrate efficacy of the CYD dengue vaccine in subcategories of VCD presented hereafter. Even though an objective of the meta-analysis is to improve the precision of the estimates for these endpoints, the number of VCD cases on these subcategories might remain limited, so results have to be interpreted with caution. In addition, the number of VCD cases on these subcategories is even more limited when considering the PD3 period. Therefore, results in CYD14 and CYD15 as well as the meta-analysis in the PD3 period are not further described for other objectives. However, conclusions in the PD3 period were comparable to those on the whole Active Phase period described hereafter, with the above mentioned limitations.

1. VE against Clinically Severe (IDMC) VCD Cases

VE against clinically severe VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASE is presented in Figure 17.

Figure 17: Forrest plot for VE against clinically severe (IDMC assessment) VCD cases during the whole active Phase due to any of the 4 serotypes - FASE



The numerator is the number of subjects with a symptomatic VCD episode in the considered period. The denominator is the number of subjects. VE of a study is calculated using density incidence: cases per 100 person-years at risk. Integrated VE and CIs are calculated using Cox regression model.

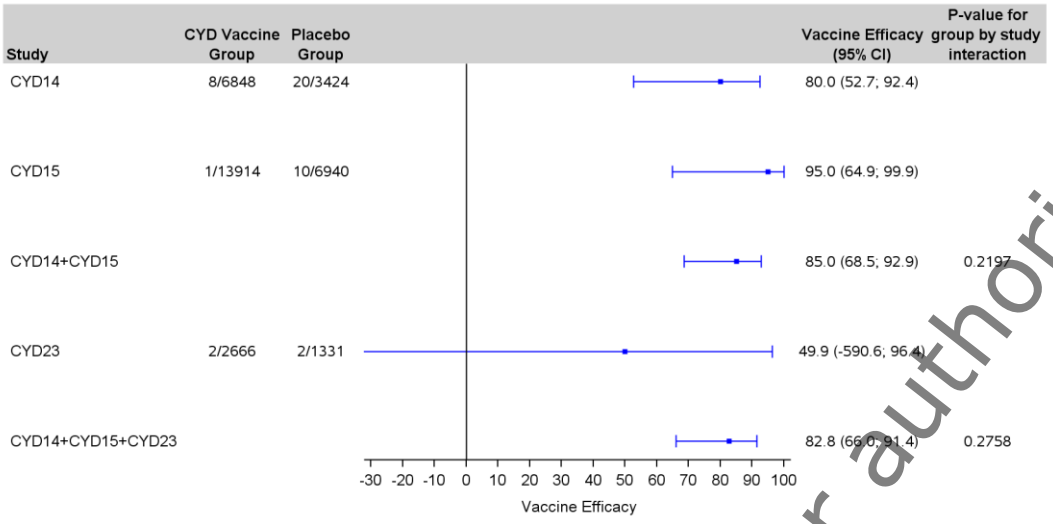
VE against clinically severe VCD cases (according to IDMC) due to any serotype was demonstrated in the 2 pivotal efficacy studies during the whole Active Phase period. In pooled analysis of CYD14 + CYD15, VE against clinically severe dengue was 79.1% (95%CI: 60.0-89.00), a value similar to that observed for VE against VCD cases.

VE against clinically severe VCD cases during the whole Active Phase against each serotype was consistent in the 2 pivotal efficacy studies for each serotype. The meta-analysis improved precision of results: the number of subjects reporting clinically severe VCD cases due to each serotype was 18 subjects for serotype 1, 11 subjects for serotype 2, 8 subjects for serotype 3 and 8 subjects for serotype 4. In the pooled analysis of CYD14 + CYD15, the VE estimate against each serotype was 75.3% (95% CI: 34.1; 90.7) for serotype 1, 81.3 (95% CI: 29.7; 95.1) for serotype 2, 83.4% (95% CI: 17.7; 96.6) for serotype 3 and 83.4% (95% CI: 18.0; 96.7) for serotype 4. However, due to the low number of cases, the VE determined should be interpreted with caution.

2. VE against DHF (WHO) VCD Cases

VE against VCD cases that met WHO criteria for DHF during the whole Active Phase due to any of the 4 serotypes in the FASE is presented in Figure 18. The numerator is the number of subjects with a symptomatic VCD episode in the considered period. The denominator is the number of subjects. VE of a study is calculated using density incidence: cases per 100 person-years at risk. Integrated VE and CIs are calculated using Cox regression model.

Figure 18: Forrest plot for VE against VCD cases that met WHO criteria for DHF during the whole Active Phase due to any of the 4 serotypes – FASE

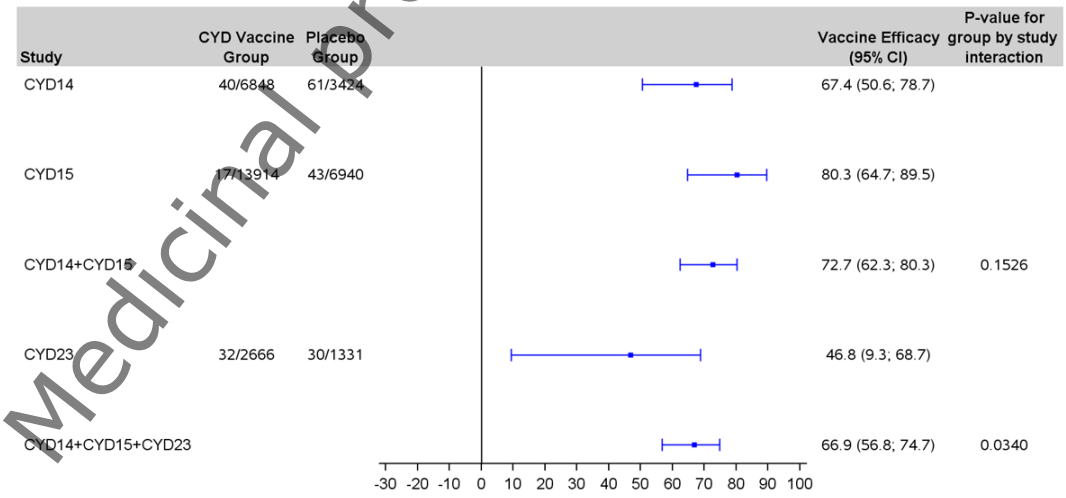


VE against WHO DHF VCD cases due to any serotype was demonstrated in the 2 pivotal efficacy studies during the whole Active Phase period in a population aged 2 to 16 years living in dengue endemic regions. The estimates determined were in the range to those obtained for prevention of clinically severe (IDMC) and prevention of VCD cases. A similar trend of efficacy level against WHO DHF VCD cases was observed for the 4 serotypes.

3. VE against Hospitalized VCD Cases

VE against hospitalized VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASE is presented in Figure 19. The numerator is the number of subjects with a symptomatic VCD episode in the considered period. The denominator is the number of subjects. VE of a study is calculated using density incidence: cases per 100 person-years at risk. Integrated VE and CIs are calculated using Cox regression mode.

Figure 19: Forrest plot or VE against hospitalised VCD cases during the whole Active Phase due to any of the 4 serotypes - FASE



VE against hospitalized VCD cases was observed in the 2 pivotal efficacy studies for each of the 4 serotypes. Although results were inconclusive for serotype 2 in CYD14 (with the lower bounds of the 95%

CI below 0), the 2 pivotal efficacy studies had consistent results for each serotype. The meta-analysis improved precision of results: the number of subjects reporting hospitalized VCD cases due to each serotype was 61 subjects for serotype 1, 49 subjects for serotype 2, 32 subjects for serotype 3 and 20 subjects for serotype 4. The pooled analysis of CYD14 + CYD15 VE estimate against each serotype was 72.1% (95% CI: 52.9; 83.4) for serotype 1, 65.7% (95% CI: 39.3; 80.6) for serotype 2, 77.4% (95% CI: 52.2; 89.3) for serotype 3 and 83.5% (95% CI: 54.5; 94.0) for serotype 4.

In conclusion, the CYD dengue vaccine reduced the occurrence of any hospitalized VCD cases during the whole Active Phase in a population aged 2 to 16 years living in dengue endemic regions. A similar trend of efficacy level against hospitalized VCD cases was observed for the 4 serotypes. VE estimates were in line with those obtained for VCD and severe cases.

Relative risk (RR) of hospitalized and severe VCD in the entire study

RR of hospitalised VCD

The RR between study groups was calculated as the ratio of the annual incidence rate (IR) in the CYD Dengue Vaccine Group to the Control Group. A RR ≤1 denotes that there was no increased risk of hospitalized VCD cases in the CYD Dengue Vaccine Group. On the other hand, a RR >1 denotes an increased risk of hospitalized VCD in subjects who received the CYD dengue vaccine. In addition, RR are presented if they can be calculated, i.e., if the number of cases in the Control Group was > 0. In addition, the number of cases is sometimes small and CIs are large, so the interpretation of the RR should be made cautiously.

Cumulatively, the risk of hospitalized dengue is higher in vaccinated children <6 years of age compared to placebo through the entire study, but an impact of immune serostatus at baseline was not determined. Hence, it is not clear if the vaccine could be beneficial to children younger than 6 years who are seropositive at the time of vaccination.

Cumulatively, the risk of hospitalized dengue is lower in vaccinated children >6 years of age compared to placebo through the entire study, but during the Hospital phase vaccinated subjects are at higher risk of hospitalized dengue than the control group that did not receive the vaccine. However subsequent data showed that this increased risk was associated with seronegative dengue serostatus at baseline (see LTFU page 140 and NS1 supplemental study page 132).

Relative risk of hospitalized severe VCD in the entire study

Although VE against severe VCD is demonstrated during the Active phase, the follow-up data up until Year 3 are inconclusive (see also LTFU). The studies had very low power to estimate the RR of (severe) hospitalised dengue according to age and dengue immune status at baseline.

Comparison of Efficacy Results According to Covariates

VE against VCD cases was further evaluated according to the following covariates:

- By age: comparison of VE in children 2 to 5 years, children 6 to 11 years and adolescents 12 to 16 years.
- By baseline dengue and FV immune status: VE in dengue immune subjects at baseline compared to dengue non-immune subjects at baseline and potential impact of JE in AP and of YF in LatAm on VE.
- By gender: comparison of VE in female and male subjects.

- In addition, VE against subcategories of VCD cases, i.e. clinically severe, WHO DHF and hospitalized VCD cases, were further evaluated according to age, baseline dengue and FV immune status and by gender.

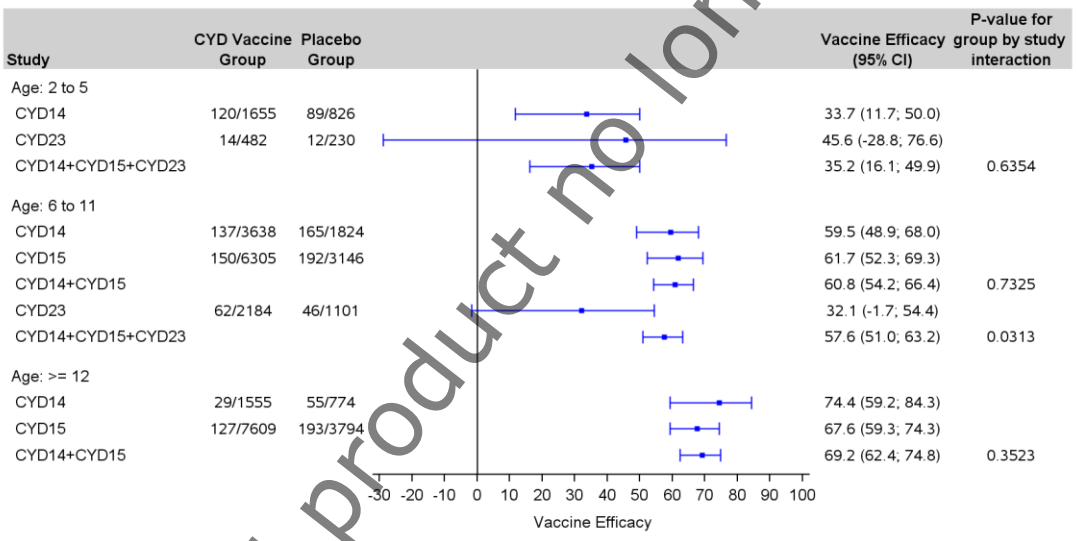
As for other objectives, the efficacy studies were not designed to demonstrate the efficacy of the CYD dengue vaccine according to covariates presented hereafter. The number of VCD cases according to each covariate might be limited, so results must be interpreted with caution.

The results are again presented first on the whole Active Phase period to increase the precision of the analyses. Results on CYD14 and CYD15 as well as the meta-analysis in the PD3 period are not further described for covariates. However, conclusions in the PD3 period were comparable to those on the whole Active Phase period described hereafter, with limitations on the number of dengue cases in the PD3 period as compared with the whole Active Phase.

1. VE Against Any VCD Cases by age

VE was evaluated first in the different age groups and then with age as a continuous variable. VE against VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASE is presented by age group in Figure 20.

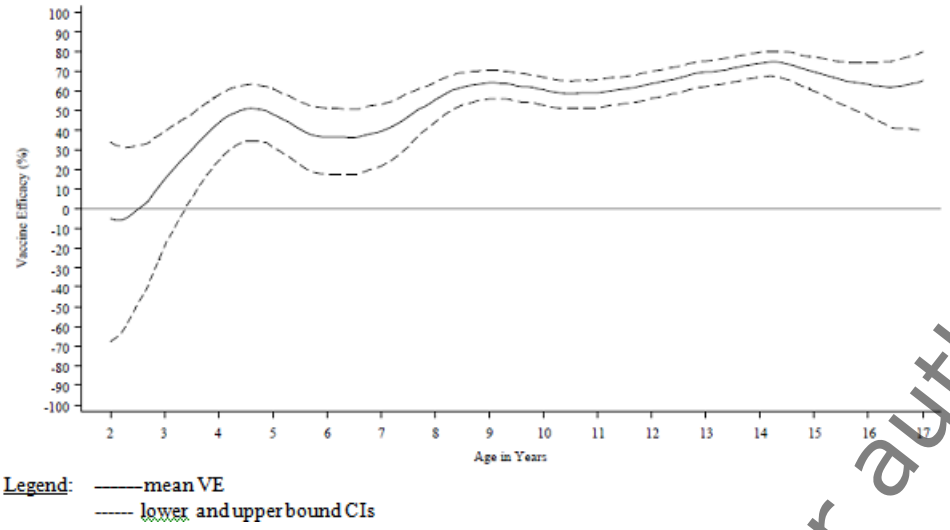
Figure 20: Forrest plot for VE against symptomatic VCD during the whole Active Phase due to any of the 4 serotypes according to age group by study– FASE



In CYD14, 209 children 2 to 5 years reported VCD cases. VE estimate for children 2 to 5 years was 33.7% (95% CI: 11.7; 50.0), with the lower bound of the 95% CI above 0. In children 6 to 11 years, the 2 pivotal efficacy studies had consistent results. The pooled analysis of CYD14 + CYD15 VE estimate in children 6 to 11 years was similar to the individual study results, with 60.8% (95% CI: 54.2; 66.4). In adolescents 12 to 16 years, the 2 pivotal efficacy studies also had consistent results. The pooled analysis of CYD14 + CYD15 VE estimate in children 12 to 16 years was similar to the individual study results, 69.2% (95% CI: 62.4; 74.8). The VE estimate against symptomatic VCD during the whole active phase due to any serotype in children ≥9YOA in individual studies (FASE) was 67.8% (95% CI 57.7, 75.6) in CYD14 and 64.7% (95%CI 58.7, 69.8) in CYD15.

The impact of age on VE was also illustrated by the kernel smoothing curve, presenting the VE according to age as a continuous variable (see Figure 21).

Figure 21: VE against symptomatic VCD cases during the whole active phase due to any of the 4 serotypes according to age using kernel smoothing – CYD14, CYD15 and CYD23 - FASE



When considering age continuously, the VE in younger subjects was the lowest, with a trend to an increased VE followed by stabilization in older children (from 9 YOA) and adolescents. These results are consistent with the observed VE in each age group.

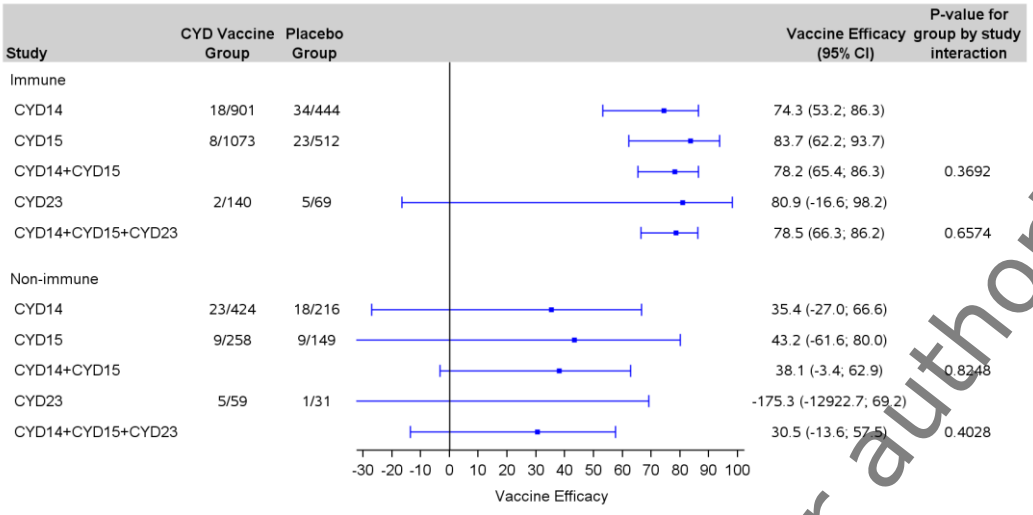
Overall, the CYD dengue vaccine reduced the occurrence of any VCD cases during the whole Active Phase period in the different age groups, with a trend toward increasing efficacy with age. It is difficult to dissociate the impact of age from baseline dengue status as these 2 variables are tightly linked together.

2. VE by Baseline Dengue immune status (Immunogenicity Subsets)

At baseline, neutralizing Abs against dengue and other FV (see below) were measured only in the subjects from the immunogenicity subset, i.e. the FASI, which represented 2000 randomized subjects each in CYD14 and CYD15 and 300 first randomized subjects in CYD23. The number of VCD cases in the FASI is therefore limited, so results must be interpreted with caution.

In CYD14, 68.1% of subjects were dengue immune at baseline. In CYD15, 80.7% of subjects were dengue immune at baseline. VE against VCD cases during the whole Active Phase due to any of the 4 serotypes in the FASI (immunogenicity subset) is presented by dengue immune status at baseline in Figure 22.

Figure 22: Forrest plot for VE against symptomatic VCD cases during the whole Active Phase due to any of the 4 serotypes according to dengue immune status at baseline by study– FASI



In baseline dengue immune subjects, the 2 pivotal efficacy studies had consistent results. The pooled analysis of CYD14 + CYD15 VE estimate in baseline dengue immune subjects was similar to the individual study results, with 78.2% (95% CI: 65.4; 86.3).

Results in dengue non-immune subjects were measurable but inconclusive because of the limited number of VCD cases. Since most subjects were dengue immune at baseline, the analysis was based on 640 subjects in CYD14 and 407 subjects in CYD15. In CYD14, 41 baseline dengue non-immune subjects reported VCD cases. VE estimate for baseline dengue non-immune subjects was 35.4% (95% CI: -27.0; 66.6), with the lower bound of the 95% CI below 0. In CYD15, 18 baseline dengue non-immune subjects reported VCD cases: 9/258 in the Dengue Group and 9/149 in the Control Group. VE estimate for baseline dengue non-immune subjects was 43.2% (95% CI: -61.6; 80.0), with the lower bound of the 95% CI below 0.

Despite inconclusive results in baseline dengue non-immune subjects (lower bound < 0), the 2 pivotal efficacy studies had consistent measurable results. Results on the pooled analysis of CYD14 + CYD15 in baseline dengue non-immune subjects were similar to the individual study results but remained inconclusive: 38.1% (95% CI: -3.4; 62.9), with the lower bound of the 95% CI below 0. In addition 95% CI of immune and non-immune subjects do not overlap.

Overall, the CYD dengue vaccine reduced the occurrence of any VCD cases during the whole Active Phase period in the baseline dengue immune population aged 2 to 16 years living in dengue endemic regions. Results in dengue non-immune subjects were measurable but inconclusive because of the limited number of VCD cases.

3. VE by Other FV Immune Status at Baseline (Immunogenicity Subsets)

In CYD14, 53.2% of subjects were JE immune at baseline and 79.1% of subjects were FV immune at baseline. In CYD15, 80.5% of subjects were YF immune at baseline and 86.8% of subjects were FV immune at baseline.

There is a known cross-reactivity of the FV PRNT assay with other FVs like dengue virus. Therefore, to describe the potential impact of prior exposure to JE or YF, only VCD cases in baseline dengue non-immune subjects should be considered. However, this analysis in the FASI would be meaningless

since the number of cases is too limited to conclude. Therefore, the standard approach in both dengue immune and non-immune subjects is used, but results must be interpreted with caution.

In CYD14, the RR of symptomatic VCD cases due to any serotype by JE immune status during the whole Active Phase was: 0.313 (95% CI: 0.16; 0.60) in baseline JE immune subjects (based on 43 VCD cases) and 0.469 (95% CI: 0.26; 0.85) in baseline JE non-immune subjects (based on 50 VCD cases). A reduction of VCD cases due to any serotype was observed in both populations, with a RR below 1.

In CYD15, the RR of symptomatic VCD cases due to any serotype by YF immune status during the whole Active Phase was: 0.181 (95% CI: 0.07; 0.40) in baseline YF immune subjects (based on 33 VCD cases) and was inconclusive with 0.510 (95% CI: 0.17; 1.56) in baseline YF non-immune subjects (based on 16 VCD cases). A reduction of VCD cases due to any serotype was observed in YF immune subjects, with a RR below 1. Results for YF non-immune subjects were inconclusive, with RR including 1.

These results are difficult to interpret due to the small number of cases and that dengue immune subjects at baseline were not excluded, i.e. dengue acts as a confounding factor. A potential effect of prior exposure to either JE or YF on the reduction of VCD cases could not be clearly established nor ruled out.

4. VE by Gender

Overall, the CYD dengue vaccine reduced the occurrence of any VCD cases in both male and female subjects. Although pooled CYD14+CYD15 VE estimates tended to be higher in male subjects (64.0% (95% CI: 57.8; 69.2)) than in female subjects (56.4% (95% CI: 49.0; 62.7), CIs were widely overlapping. Therefore, it is difficult to reach a conclusion regarding the potential impact of gender on the VE of the CYD dengue vaccine.

5. VE against Clinically Severe, WHO DHF and Hospitalized VCD cases according to covariates

The purpose of these analyses was to address potential observations on subcategories of VCD cases. Due to the low number of VCD cases observed in these subcategories of the subjects, none of these analyses could be used for any demonstration. Compared to all VCD cases, the same trends were observed on these specific endpoints.

Comparison of Efficacy Results in the 9-16YOA Population

Overall, the observations made on the overall study population are similar in the 9-16YOA population. However, the VE in subjects from 9 to 16 years in endemic regions tended to be higher than the VE in the overall efficacy population against any VCD cases, any clinically severe, WHO DHF and hospitalized VCD cases. Younger subjects from 2 to 8 years for whom a lower VE was observed on the analysis of VE in the whole efficacy population are excluded from the indication.

The pooled analysis of CYD14+CYD15 VE estimate against symptomatic VCD during the whole active phase was 65.6% (95%CI: 60.7; 66.9). As for the overall population, VE for each of the 4 serotypes in subjects from 9 to 16 years in the 2 pivotal efficacy studies during the whole Active Phase period varied across serotype, with a lower VE for serotypes 1 and 2 as compared with serotypes 3 and 4. The pooled analysis of CYD14 + CYD15 VE estimate against clinically severe VCD cases in subjects from 9 to 16 years was 93.2% (95% CI: 77.3; 98.0). The 2 pivotal efficacy studies had consistent results. The pooled analysis of CYD14 + CYD15 VE estimate against hospitalized VCD cases in subjects from 9 to 16 years was similar to the individual study results, with 80.8% (95% CI: 70.1; 87.7).

The 2 pivotal efficacy studies had consistent results regarding VE estimate in subjects from 9 to 16 years in relation to baseline dengue immune status, showing again that VE was higher in the dengue

seropositive subjects at baseline. In fact, the pooled analysis of CYD14 + CYD15 VE estimate in baseline dengue immune subjects was 81.9% (95% CI: 67.2; 90.0), and in baseline dengue non-immune subjects 52.5% (95% CI: 5.9; 76.1). Importantly, in the two individual studies the lower bound of the 95% CI of the VE included zero, hence no conclusion can be drawn on efficacy in baseline dengue seronegative subjects.

A summary of the results in the 9-16 YOA population on the main endpoints is included in Table 20 below:

Table 20: VE against symptomatic, Hospitalised or Severe VCD over the 25-month period after the first injection in subjects 9 to 16 years of age (FASE)

	CYD14 VE % (95% CI)* N=3316	CYD15 VE % (95% CI)* N=13 914	Pooled CYD14+CYD15 VE % (95% CI)* N=17 230	CYD23 VE % (95%CI)* N = 1032	Pooled*** CYD14+CYD15+CYD23 VE % (95% CI)* N = 18262
Symptomatic VCD Any serotype	67.8 (57.7; 75.6)	64.7 (58.7; 69.8)	65.6 (60.7; 69.9)	43.3 (-18.9 ; 72.7)	64.9 (60.0 ; 69.2)
Serotype 1	65.7 (46.6; 78.2)	54.8 (40.2; 65.9)	58.4 (47.7; 66.9)	78.4 (5.4 ; 96.4)	59.2 (48.9 ; 67.4)
Serotype 2	36.8 (-10.1; 63.3)	50.2 (31.8; 63.6)	47.1 (31.3; 59.2)	5.9 (-178.6 ; 65.1)	44.6 (28.9 ; 56.9)
Serotype 3	69.5 (31.9; 87.0)	74.2 (63.9; 81.7)	73.6 (64.4; 80.4)	-1.2 (-5870.2 ; 94.7)	73.0 (63.7 ; 79.9)
Serotype 4	87.9 (75.5; 94.6)	80.9 (70.9; 87.7)	83.2 (76.2; 88.2)	100.0 (-1873.3 ; 100.0)	83.3 (76.4 ; 88.2)
Any serotype in subjects dengue immune prior to vaccination **	79.2 (47.2; 92.7)	83.7 (62.2; 93.7)	81.9 (67.2; 90.0)	NC (NC)	81.9 (67.2 ; 90.0)
Any serotype in subjects dengue non-immune prior to vaccination **	61.6 (-21.1; 88.1)	43.2 (-61.6; 80.0)	52.5 (5.9; 76.1)	NC (NC)	49.2 (0.4 ; 74.1)
Hospitalised VCD†	81.6 (60.7; 92.0)	80.3 (64.7; 89.5)	80.8 (70.1; 87.7)	72.5 (19.0 ; 91.7)	79.7 (69.6 ; 86.5)
Clinically severe VCD cases†	90.9 (58.4; 99.0)	95.5 (68.8; 99.9)	93.2 (77.3; 98.0)	49.4 (-3870.3 ; 99.4)	91.3 (74.9 ; 97.0)
DHF meeting any WHO criteria	90.9 (58.4; 99.0)	95.0 (64.9; 99.9)	92.9 (76.1; 97.9)	100.0 (-1871.5 ; 100.0)	93.2 (77.3 ; 98.0)

N: number of subjects per study. * The efficacy of Dengvaxia is considered as significant as the lower bound of the 95% CI is greater than 25% (CYD14 and CYD15) or greater than 0% (CYD23). CI: confidence interval. **Vaccine efficacy analyses according to dengue serostatus measured by PRNT50 test at baseline (before the first injection) were performed in the immunogenicity subset of 2000 subjects each in CYD14 and CYD15 and 300 subjects in CYD23. *** Pooled results of CYD14, 15 and 23 need to be interpreted cautiously because of differences in the Dengue confirmatory test and acute febrile illness definition between CYD14/15 and CYD23. †The efficacy against hospitalised and severe VCD was not a primary objective and cut-off thresholds to define statistical significance were not pre-specified.

Clinical efficacy data for subjects 9 to 16 years of age in endemic areas, dengue seropositive at baseline

VE results according to the primary endpoint (symptomatic VCD cases occurring during PD3 period) in subjects 9 to 16 years of age, seropositives at baseline are shown in Table 21 for the immunosubset of studies CYD14, CYD15 and CYD23.

Table 21: VE against symptomatic VCD cases over the 12-month period starting from 28 days after the third injection due to any of the 4 serotypes in dengue seropositive subjects 9 to 16 years

	CYD14		CYD15		CYD23		Pooled CYD14+CYD15		Pooled * CYD14+CYD15+ CYD23	
	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group
Cases / person-years	4/471	9/241	7/1002	17/472	0/55	0/19	11/1473	26/713	11/1528	26/732
VE % (95%CI)	77.2 (18.3; 94.9)		80.6 (50.7; 93.2)		NC		79.4 (58.4; 89.8)		79.4 (58.4; 89.8)	

N: number of subjects per study
Cases: number of subjects with at least one symptomatic virologically-confirmed dengue episode in the considered period.
Person-years: sum of time-at-risk (in years) for the subjects during the study period.
CI: confidence interval.
NC: Not computed (the absence of cases in vaccine and control group does not permit to calculate VE nor CI)
*Pooled results of CYD14, 15 and 23 need to be interpreted cautiously because of differences in the Dengue confirmatory test and acute febrile illness definition between CYD14/15 and CYD23.

VE against symptomatic VCD during the whole active phase in subjects 9 to 16 years of age dengue seropositive at baseline and for the immunogenicity subset for pooled CYD14+CYD15+CYD23 is estimated at 81.9% (95% CI: 67.2 ; 90.0).

In subjects 9-16YOA dengue seropositive at baseline (immunogenicity subset), one clinically severe VCD case and one WHO DHF VCD case was reported during the active phase in the control group in each individual study (CYD14 and CYD15) versus none in the vaccine group. Four hospitalized VCD cases in CYD14 and two hospitalized VCD cases in CYD15 were reported in the control group versus none in the vaccine group. These data are inconclusive due to the low number of cases in the immunogenicity subset. However, the extrapolated vaccine efficacy (1- Hazard Ratio), obtained from an exploratory analysis (pooled CYD14 + CYD15 + CYD23) over 25-month period after the first injection, is estimated at 89.2% (95% CI: 78.5; 94.6) for hospitalized VCD and 95.3% (95% CI: 68.9; 99.3) for severe VCD.

Efficacy summary of findings

The comparison of efficacy data from 2 pivotal efficacy studies, which included subjects from 2 to 16 years of age in AP and LatAm endemic regions, receiving 3 injections of the CYD dengue vaccine administered 6 months apart, showed that:

- Overall, the CYD dengue vaccine reduced the occurrence of any VCD cases in a population aged 2 to 16 years living in dengue endemic regions and who received the full immunization schedule.
- Although the VE varied across serotypes, with a lower VE against serotypes 1 and 2 as compared with serotypes 3 and 4, the efficacy of the CYD dengue vaccine was demonstrated against each of the 4 serotypes. The circulating serotype has an impact on the VE as illustrated by the different VE observed across countries.
- The CYD dengue vaccine reduces the occurrence of any clinically severe (IDMC) and DHF WHO VCD cases, with also a significant reduction in the duration of hospitalizations.
- The CYD dengue vaccine reduces the occurrence of hospitalized VCD cases due to any serotypes.

- The CYD dengue vaccine reduces the clinical signs of DHF such as haemorrhage, plasma leakage and thrombocytopenia.

The analyses of the covariates suggested that:

- The VE estimate was high in baseline dengue immune subjects and measurable but inconclusive in baseline dengue non-immune subjects.
- The VE estimates tended to increase with age, although it is difficult to dissociate the impact of age from the impact of the dengue immune status of the subjects at baseline.

Overall, the same conclusions regarding a reduction of any VCD cases, any clinically severe, WHO DHF and hospitalized VCD cases were observed in the claimed population in the indication, i.e. subjects from 9 to 16 years in endemic regions, with higher VE estimates.

Risk of Symptomatic VCD and Dengue Hospitalization and/or Severe Dengue According to Dengue Serostatus in CYD Vaccine Efficacy Trials

This was a supplemental analysis for the evaluation of dengue outcomes according to dengue serostatus as determined by a Dengue anti-NS1 IgG ELISA. The evaluation was based on data and blood samples collected in studies CYD14, CYD15, and CYD23/57.

During the first year of the Hospital Phase, there was an imbalance and a trend towards a higher risk of hospitalized symptomatic VCD in the youngest vaccine recipients in CYD14 (subjects aged 2 to 5 years at enrolment). This could be interpreted as a possible indication of an increased risk of dengue hospitalization or severe dengue illness in individuals who have not been exposed to dengue prior to being vaccinated with CYD dengue vaccine. This hypothesis could not be adequately evaluated with data from the CYD dengue vaccine efficacy studies, because pre-vaccination samples were only obtained for a small proportion of participants (only subjects included in the immunogenicity subsets, i.e. 10-20% of subjects from the efficacy trials) and because the incidence of dengue hospitalization or severe dengue is much lower than the incidence of any symptomatic VCD, resulting only in partial and largely imprecise estimates of the risk according to prior exposure to natural dengue infection. This was particularly true for seronegative subjects, since only approximately 26% of subjects from the immunosubsets were seronegative at baseline.

Nevertheless, because blood samples were collected for all study participants approximately 1 month after the third injection of CYD dengue vaccine or placebo (month [M] 13), classification of dengue serostatus (as a surrogate of prior natural dengue exposure) of study participants at this time-point could be used as a baseline for the evaluation of outcomes that occurred later. However, the PRNT assay (used until now for the classification of baseline dengue serostatus) is directly affected by the immune responses induced by the vaccine, i.e. a positive PRNT assay at M13 can be the result of either prior dengue exposure or CYD dengue vaccination. To overcome this challenge, the Applicant has leveraged an assay originally developed by University of Pittsburg (Pittsburg, PA, USA) and optimized by Sanofi Pasteur. This assay measures total immunoglobulin G (IgG) antibodies against the non-structural protein 1 (NS1) of the dengue virus by Enzyme-Linked Immunosorbent Assay (ELISA). Because the NS1 protein is not conserved between the dengue virus and the yellow-fever virus, previous exposure to CYD dengue vaccine is not expected to induce meaningful levels of antibody against the dengue NS1 protein.

The application of the Dengue anti-NS1 IgG ELISA assay to M13 samples is therefore useful for expanding the existing data on both VE and potential risk of dengue hospitalization and/or severe dengue according to baseline serostatus in the CYD dengue vaccine efficacy trials. Dengue serostatus, as determined by the Dengue anti-NS1 IgG ELISA assay result at M13, was utilized as a covariate to assess the effects of CYD

dengue vaccine for outcomes that occur after M13. In addition, with the multiple imputation method, outcomes for cases that occur after M0 could be assessed as well. Serostatus at M13 is assumed to serve as a surrogate of baseline serostatus before vaccination. This is particularly the case for those subjects that test seronegative at M13, who are assumed to have been seronegative at enrolment as well. A small proportion of subjects that are seropositive at M13 were expected to correspond to subjects that were actually seronegative at enrolment, but who had dengue infection between enrolment and M13. Given that the overall incidence of dengue during this period was low, this was expected to be a relatively rare occurrence.

In order to minimize misclassification of serostatus as seropositive, this supplementary study excluded cases that were diagnosed with VCD before M13 when serostatus was determined by the NS1 assay at M13. With the multiple imputation and TMLE methods, cases from M0 onwards could also be included in the analysis. The overall aim of this supplementary study was therefore to evaluate the risk of dengue-associated outcomes that occurs post-M13 in CYD dengue vaccine or control study participants according to baseline serostatus as determined by Dengue anti-NS1 IgG ELISA assay performed on M13 samples. To maximize the power of the study, data was pooled across the 3 efficacy studies. However, supportive data by study were also generated. Given that the age targeted for the vaccine indication is 9 years and older, the primary analysis focused on this age group, with supportive secondary and exploratory data also generated overall and according to additional age groups.

Assay

The assay used for this analysis was an Enzyme-Linked Immunosorbent Assay (ELISA) for the detection and quantitation of human IgG antibodies against Dengue Virus (DENV) non-structural protein 1 (NS1) in human sera. The amount of antibody bound to the DENV NS1 antigen coated microtiter plate wells was determined by a colorimetric substrate reaction after the binding of a secondary antihuman IgG antibody-enzyme conjugate. The concentration of the IgG antibodies in serum was then derived by extrapolation from a standard curve, which was generated from multiple dilutions of a reference standard serum run concurrent to the sample with an assigned concentration (in EU/mL). The optimized Dengue NS1 IgG ELISA method was qualified. It has relatively high specificity (ca. 95%) to identify seronegatives. In addition to the regular assay qualification, the Applicant performed an assay characterization study that evaluated the performance characteristics of the dengue NS1 IgG ELISA assay for the intended use, i.e. assessment of dengue serostatus on some M13 blood samples in the CYD14, CYD15, and CYD57 efficacy trials. A threshold of <9 EU/mL (the LLOQ of the method) as compared to the threshold of 20 EU/mL was recommended to result in low rate to misclassify dengue exposed as seronegative by the assay (the false positive rate 31.4% and the false negative rate was 4.7%), but results in high false positive rate (erroneous classification of samples from dengue unexposed individuals as seropositive). It is agreed that for the present study, minimising the risk of incorrect inclusion of dengue exposed participants (dengue seropositive) into the seronegative category was particularly important, especially because in endemics settings the participants had a high likelihood of prior exposure to dengue.

Competition based analysis using homologous and heterologous antigens identified the potential for interference by IgG to Zika (Uganda) NS1. However no significant (>25%) cross-reactivity was detected to any of the other recombinant Flavivirus NS1 proteins evaluated including JE, WNV, TBE, and Usutu. Regarding the potential interference of dengue NS1-specific IgG to Zika, it has to be noted that assessment of dengue serostatus was performed on M13 blood samples, which were taken before Zika epidemics in Latin America, so with no impact on the results.

Case-cohort design

The Applicant used a case-cohort design as introduced by Prentice to analyse cohort data in which occurrence of an event of interest is rare (in this case approximately 1% for the safety outcomes among all 3 studies). In the case-cohort study design, a random sample of subjects, referred to as the sub-cohort, was first chosen from the entire study population. Subjects with the event of interest but not selected in the sub-cohort were then included in the case-cohort analysis. As the sub-cohort is chosen without regard to any outcomes, it may serve as a comparison group for several different events of interest. In this supplementary study, the sub-cohort consisted of a random sample of 10% of all subjects who had a M13 visit and provided post-dose 3 (PD3) blood specimens from the studies CYD14, CYD15, and CYD23/57 (i.e. approximately 3300 subjects). This random sample initially excluded study participants with known VCD occurring between enrolment and M13, but in the final CSR the expanded case-cohort included these events allowing to estimate risk from M0 onwards. The cases, corresponding to all events of interest, were all symptomatic VCD, all hospitalized dengue, or all severe dengue, depending on the analysis.

In the original supplemental NS1 study, dengue serostatus assessed by dengue anti-NS1 IgG ELISA assay at M13 was utilized as a surrogate of baseline serostatus. Although the NS1 protein is not completely conserved between the dengue virus and the YF virus, some level of cross-reactivity was found in the original supplemental study. Data showed that seronegative vaccinated subjects had more chance than seronegative placebo subjects to be misclassified as seropositive based on the anti-NS1 titers at M13, due to the influence of the CYD vaccination on the read-out. Therefore, in the extension study presented here, the Applicant used several methods to classify baseline serostatus. The principal was based on M0 measured/imputed PRNT50 (see also below). This parameter was either measured in the immunogenicity subset or predicted based on Dengue anti-NS1 ELISA values at M13 (continuous) and other covariates such as age, sex, country, indicator of whether subject had VCD between M0 and M13, time between onset of VCD case and M13 sample collection date, and treatment group. The model used for the imputation was ascertained in the immunogenicity subset using baseline serostatus (negative or positive) as dependent variable.

In addition, complementary assessments used serostatus classification in the expanded case-cohort study based on M13 anti-NS1 readouts. Subjects were classified as dengue seropositive or seronegative based on two alternative cut-off thresholds of 9 EU/mL and 20 EU/mL.

Methods to Estimate Risk and Vaccine Efficacy (VE):

1) Principal Analyses Based on PRNT50 Baseline Serostatus

The principal analyses determined risk of dengue hospitalization/severe dengue and VE against symptomatic VCD based on PRNT50 at baseline to determine serostatus. PRNT50 baseline serostatus was either measured (for subjects in the immunogenicity subset) or predicted in subjects with missing baseline values. Prediction of PRNT50 baseline serostatus was undertaken by 2 separate methods, parametric Multiple Imputation and the non-parametric SuperLearner approach, using available M13 dengue anti-NS1 values and other covariates. For the SuperLearner method, risk and efficacy estimates were then estimated by using inverse probability weighing integrated into a Targeted Minimum Loss based Estimation (TMLE) framework.

Both methods (Multiple imputations and SuperLearner methodology) were used to estimate PRNT50 baseline serostatus for subjects in the case-cohort with missing baseline values using M13 dengue anti-NS1 and PRNT titres, age, vaccine group, sex, country, indicator of whether subject had VCD between M0 and M13, time between VCD and sample collection (if subject had an event between M0 and M13), and other variables were included as predictors.

Based on the PRNT50 baseline serostatus (measured/imputed), risk of dengue hospitalization and VE against symptomatic VCD were then estimated by using Cox regression models (with the Prentice weighting method) or the targeted minimum loss-based estimator approach. For both approaches, data up until M25 was included for efficacy analyses (M0-M25 or M13-M25). For safety analysis, all data (from M0 or M13) until data cut-off date was included:

- For TMLE analysis: until M66
- For MI analysis: until March 2017, i.e. 4 years of the Hospital Phase for CYD57; a minimum of 3 years and 3 months of Hospital Phase/Surveillance Expansion Period (SEP) surveillance for CYD14; and a minimum of 3 years of Hospital Phase/SEP surveillance for CYD15.

2) Complementary Analysis Based on M13 Measured NS1 Values as a Surrogate of Baseline Serostatus ("NS1" Analysis)

M13 dengue anti-NS1 titres were used as a surrogate of baseline serostatus before vaccination. Evaluation of the different outcomes (symptomatic VCD, hospitalized VCD, severe dengue) was performed for subjects in the case-cohort from M13 onwards (M13-M25 for efficacy analysis, M13 until cut-off date for safety analysis).

Two thresholds for seronegativity were used to categorize serostatus: <9 EU/mL and <20 EU/mL. The NS1 threshold of 9 EU/mL was defined to increase the specificity of identifying seronegatives (low false seronegativity rate). Evaluation of study estimates based on an alternative threshold of 20 EU/mL was also performed.

The risk of dengue hospitalization/severe dengue and VE against symptomatic VCD was estimated using a modified Cox regression model (with the Prentice weighting method).

3) Attributable risk Associated with the Above Safety Analyses

To better assess how these new data can be translated at the population level, the attributable risk (AR), i.e. the difference in the disease rates in subjects exposed to the vaccine and subjects unexposed to the vaccine, was calculated. The risk or benefit that is attributable to the vaccine is defined as the difference in incidence at each time-point, as follows:

AR = incidence in CYD group – incidence in placebo group

It represents the numbers of dengue hospitalizations, or severe dengue cases, that are prevented (if AR<0) or caused (if AR>0) by the vaccine in a population that has the same dengue incidence as in the clinical studies.

By contrast with relative risks, attributable risks depend on the background incidence of the condition in the population. Cumulative incidences of dengue hospitalization, or severe dengue, were extracted from the corresponding survival timetables used for Kaplan-Meier estimates. Dengue incidence was estimated overall (on the complete 5-year period) and yearly (cumulative).

Results

The MI analysis from M0 onwards, and NS1 analysis (threshold [Th] 9) from M13 onwards are presented. There was general overall consistency across the different methodologies (TMLE analysis, MI analysis from M13, NS1 Th20). Results are summarised by age group (9-16YOA as per indication, and <9YOA) and by serostatus.

1a) Estimates of Risk and Efficacy in Seronegative Individuals 9-16 Years

The risk of dengue hospitalization and the risk of severe dengue (hazard ratios [HR]), and vaccine efficacy against symptomatic VCD in subjects 9-16 years at enrolment and classified as seronegative by PRNT50 at M0 (imputed/predicted, MI analysis) or by NS1 at M13 (threshold 9) are presented for all studies in Figure 23.

Figure 23: Estimates of risk of hospitalized and severe dengue and efficacy against symptomatic VCD in seronegative subjects 9-16 years.

Serostatus classification Time period	CYD group n	CYD group N	Control group n	Control group N	Subjects 9-16 years at enrollment and classified as seronegative	p-value
					Risk of dengue hospitalization - CYD14+CYD15+CYD23/57 (HR and 95% CI)	
MI M0 onwards	64.2	375.1	25.3	207.2	0.743 — 1.412 — 2.682	0.287
NS1 Th 9 M13 onwards	56	330	20	171	0.851 — 1.455 — 2.488	0.171
					0.1 Favourable 1 Unfavourable 10	
					Risk of severe dengue (IDMC) - CYD14+CYD15+CYD23/57 (HR and 95% CI)	
MI M0 onwards	14.8	375.1	3.6	207.2	0.472 — 2.435 — 12.559	0.283
NS1 Th 9 M13 onwards	12	330	1	171	0.809 — 6.251 — 46.322	0.079
					0.1 Favourable 1 Unfavourable 10 100	
					Vaccine efficacy (VE) against symptomatic VCD - CYD14 +CYD15 (VE and 95% CI)	
MI M0- M25	174.3	353.6	148.9	193.7	Unfavourable Favourable -0.009 — 0.388 — 0.629	0.054
NS1 Th 9 M13-M25	104	309	62	157	-0.179 — 0.18 — 0.43	0.284
					-0.2 0 0.2 0.4 0.6 0.8 1	

n: number of subjects fulfilling the item listed
N: total number of subjects selected in sub-cohort
NS1 analysis: subjects with VCD cases before M13 are excluded from the analyses
MI analysis: n and N are average numbers from 10 iterations of multiple imputations
Study group classified as treated (Subjects classified as CYD Dengue Vaccine Group if received at least 1 injection of CYD dengue vaccine)

The HR against dengue hospitalization and severe dengue in seronegative subjects 9-16 years at enrolment was >1 for both methods. Although not statistically significant, these results show an increased risk of dengue hospitalization and severe dengue in seronegative subjects 9-16 years at enrolment in the pool of CYD14+CYD15+CYD23/57.

The analysis of HR against hospitalized VCD cases by time periods (Active Phase [up to M25], Y1 of Hospital Phase, Y2 of Hospital Phase, and beyond Y2 of Hospital Phase), showed that during the Active Phase, risk of hospitalized dengue was close to 1 although not statistically significant in the seronegative subjects 9-16 years of age. However, an increased risk of hospitalized VCD cases was observed over the entire duration of the Hospital Phase.

When considering the number of cases of severe dengue, with the NS1 method (threshold 9), 12 cases of severe dengue were observed in the CYD dengue vaccine group compared to 1 severe case in the placebo group. Clinical signs and symptoms of hospitalized and severe VCD cases in seronegative individuals 9-16 years was comparable between the CYD dengue vaccine group and the placebo group.

The pooled analysis of CYD14+CYD15 VE estimate against VCD cases in seronegative subjects 9-16 years was 18% for the NS1 Th 9 analysis (M13-M25) and 38% for the MI analysis (M0-M25) but with a lower bound of the CI below 0 (see figure above).

When looking at dengue hospitalization by time period in seronegative subjects aged 9-16 years, for all the methods used, there is a limited and non-statistically significant trend toward a protective effect of CYD during the Active Phase (two years post-vaccination 1). The relative risks of hospitalized dengue were overall close to 1 and not statistically significant. Point estimates of the RR are 0.57 and 0.84 (respectively for TMLE and MI) over the pooled studies. The HR was > 1 during the Active Phase when estimated with anti-NS1 M13 (Threshold 9) to classify serostatus.

The excess risk associated with vaccination appears during Year 3, and is pronounced during that year (relative risk point estimate ranging from 2.41 to 2.89 depending on the imputation method), and then decreases on Year 2 of the HP and beyond although remaining above 1.00.

The relative risk estimates were higher for CYD15 compared to CYD14. A statistically significant increase was observed in Y1 of Hospital Phase (Y3) in CYD15 using the MI M0 approach (HR: 6.15 [95% CI: 1.12, 33.67] and with anti-NS1 Threshold 9 EU/mL (HR: 7.76 [95% CI: 2.88, 20.91]. The HR remained >1 in Y2 and beyond Y2 of the Hospital Phase, although the risk was not statistically significant in these two time periods. With the exception of the latter result in the CYD15, none of the association was statistically significant.

Kaplan Meier analysis suggests that the risk of naïve CYD exceeds the risk of naïve Placebo subjects from around M30.

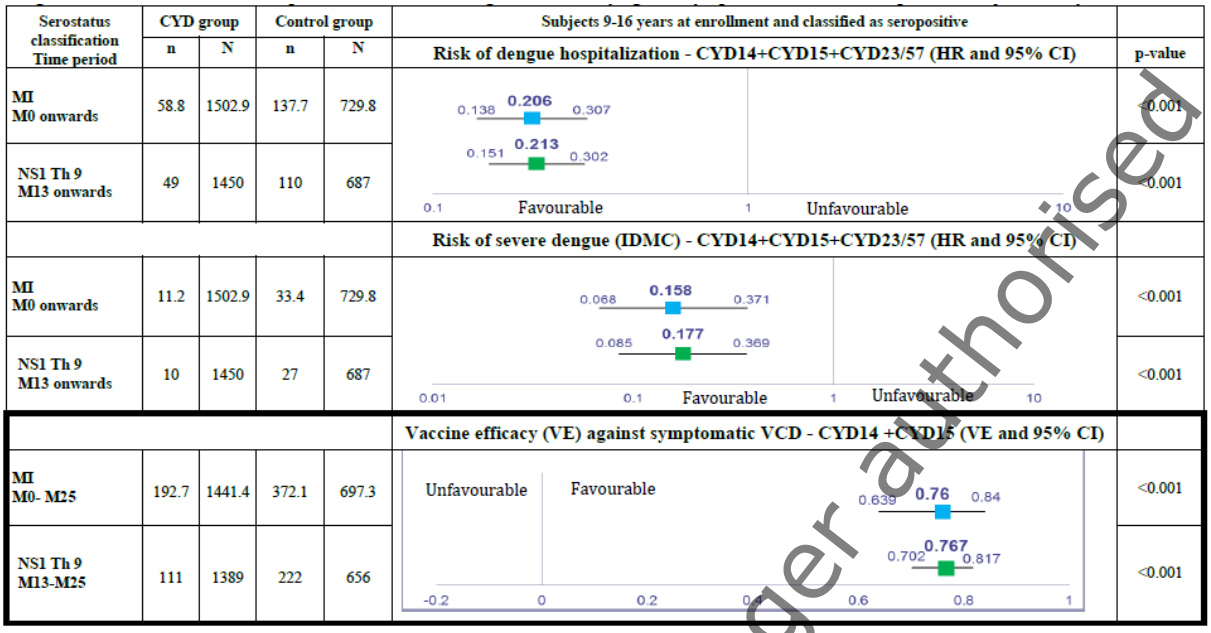
Based on sensitivity analyses, for severe dengue the HR pooled from all studies (CYD14, CYD15, CYD57) in seronegative subjects aged 9-16 years was 2.43 (95% CI: 0.47, 12.56) for the MI M0 approach. In the pooled analysis of studies (CYD14 + CYD15 + CYD57), the HR/RR were >1 across both TMLE and MI methods and ranged from 1.41 (TMLE M0) to 3.08 (MI M13). The HR was 6.25 (95% CI: 0.81, 48.32, p = 0.079) in subjects classified as seronegative by anti-NS1 at M13 (Threshold 9). Although the HR/RR was greater than 1, the risk estimated did not reach statistical significance by any methods.

1b) Estimates of Risk and Efficacy in Seropositive Individuals 9-16 Years

The risk of dengue hospitalization, the risk of severe dengue, and vaccine efficacy against symptomatic VCD in subjects 9-16 years at enrolment and classified as seropositive by PRNT50 at M0 (imputed/predicted, MI analysis) or by NS1 at M13 (threshold 9) are presented for all studies in Figure 24.

Figure 24: Estimates of risk of hospitalized and severe dengue and efficacy against symptomatic VCD in seropositive subjects 9-16 years

Serostatus classification Time period	CYD group n	CYD group N	Control group n	Control group N	Subjects 9-16 years at enrollment and classified as seropositive	p-value
					Risk of dengue hospitalization - CYD14+CYD15+CYD23/57 (HR and 95% CI)	
MI M0 onwards	58.8	1502.9	137.7	729.8	0.206 (0.138 – 0.307)	<0.001
NS1 Th 9 M13 onwards	49	1450	110	687	0.213 (0.151 – 0.302)	<0.001
					Risk of severe dengue (IDMC) - CYD14+CYD15+CYD23/57 (HR and 95% CI)	
MI M0 onwards	11.2	1502.9	33.4	729.8	0.158 (0.068 – 0.371)	<0.001
NS1 Th 9 M13 onwards	10	1450	27	687	0.177 (0.085 – 0.369)	<0.001
					Vaccine efficacy (VE) against symptomatic VCD - CYD14 +CYD15 (VE and 95% CI)	
MI M0- M25	192.7	1441.4	372.1	697.3	0.76 (0.639 – 0.84)	<0.001
NS1 Th 9 M13-M25	111	1389	222	656	0.767 (0.702 – 0.817)	<0.001



n: number of subjects fulfilling the item listed
N: total number of subjects selected in sub-cohort
NS1 analysis: subjects with VCD cases before M13 are excluded from the analyses
MI analysis: n and N are average numbers from 10 iterations of multiple imputations
Study group classified as treated (Subjects classified as CYD Dengue Vaccine Group if received at least 1 injection of CYD dengue vaccine)

The HR against dengue hospitalization and severe dengue in seropositive subjects 9-16 years at enrolment was <1 and statistically significant for both methods in the pool of CYD14+CYD15+CYD23/57. These results showed a decreased risk of dengue hospitalization and of severe dengue in seropositive subjects 9-16 years at enrolment in each of the 3 efficacy studies.

The pooled analysis of CYD14+CYD15 VE estimate against VCD cases (M0-M25 or M13-M25) in seropositive subjects 9-16 years was consistent across the 2 methods and showed a statistically significant VE of approximately 76%. Significant vaccine efficacy was also observed in individual studies.

1c) Attributable risk in 9-16 Years Olds

The attributable risk/benefit for seropositives aged 9-16 years at M0, with serostatus based on PRNT50 at M0 (PRNT50), was calculated during M0-M61 period using MI approach. The results show that in subjects classified as seropositive (i.e. subjects already exposed to dengue based on PRNT50 test with MI approach), aged 9-16 years, about 15 hospitalized dengue cases, or 4 severe dengue cases, could be prevented per 1000 vaccinees during 5 years of follow up from the first injection. These results were obtained in a population that had, in non-vaccinated subjects classified as previously exposed to dengue, a cumulative incidence of 1.89% for hospitalized dengue cases over 5 years, and 0.48% for severe dengue cases over 5 years.

In subjects classified as seronegative (i.e. with no previous dengue infection detected via PRNT50 test with MI approach), aged 9-16 years, it was estimated that during a 5 year follow-up period, about 5 additional hospitalized dengue cases, or 2 additional severe dengue cases per 1000 vaccinees could occur following vaccination. These results were obtained in a population that had, in non-vaccinated subjects classified as not previously exposed to dengue, a cumulative incidence of 1.09% for hospitalized dengue cases over 5 years, and 0.17% for severe dengue cases over 5 years. In such a population, the estimates

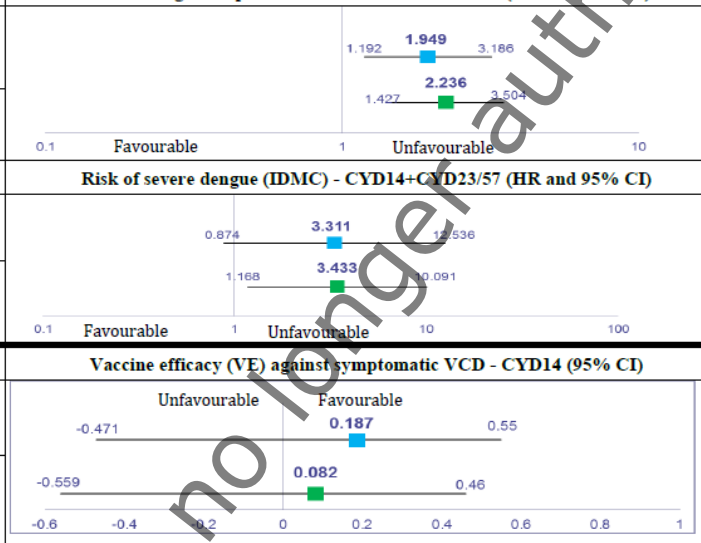
from the long-term analysis suggest that the onset of increased risk was mainly during the third year following the first injection.

2a) Estimate of Risk and Efficacy in Seronegative Individuals 2-8 Years

The risk of dengue hospitalization, the risk of severe dengue, and vaccine efficacy against symptomatic VCD in subjects 2-8 years at enrolment and classified as seronegative by PRNT50 at M0 (imputed/predicted, MI analysis) or by NS1 at M13 (threshold 9) are presented for all studies in figure 23.

Figure 25: Estimates of risk of hospitalized and severe dengue and efficacy against symptomatic VCD in seronegative subjects 2-8 years.

Serostatus classification Time period	CYD group n	CYD group N	Control group n	Control group N	Subjects 2-8 years at enrollment and classified as seronegative	p-value
					Risk of dengue hospitalization - CYD14+CYD23/57 (HR and 95% CI)	
MI M0 onwards	137.4	192.8	37.2	100.6	1.949 (1.192, 3.186)	0.008
NS1 Th 9 M13 onwards	131	182	33	101	2.236 (1.427, 3.504)	<0.001
					Risk of severe dengue (IDMC) - CYD14+CYD23/57 (HR and 95% CI)	
MI M0 onwards	30.1	192.8	5	100.6	3.311 (0.874, 12.536)	0.077
NS1 Th 9 M13 onwards	25	182	4	101	3.433 (1.168, 10.091)	0.025
					Vaccine efficacy (VE) against symptomatic VCD - CYD14 (95% CI)	
MI M0- M25	107.5	141.7	69.7	74.5	0.187 (-0.471, 0.55)	0.484
NS1 Th 9 M13-M25	49	128	29	73	0.082 (-0.559, 0.46)	0.75



n: number of subjects fulfilling the item listed
N: total number of subjects selected in sub-cohort
NS1 analysis: subjects with VCD cases before M13 are excluded from the analyses
MI analysis: n and N are average numbers from 10 iterations of multiple imputations
Study group classified as treated (Subjects classified as CYD Dengue Vaccine Group if received at least 1 injection of CYD dengue vaccine)

The HR against dengue hospitalization and severe dengue in seronegative subjects 2-8 years at enrolment was >1 and was statistically significant for both methods (only for the NS1 Th9 method for severe dengue). For subjects classified as seronegative by anti-NS1 at M13 (Threshold 9), a statistically significant imbalance in the number of severe cases between treatment groups was observed. There were 25 severe cases observed in the CYD Dengue Vaccine Group and 4 cases in the Placebo group (HR: 3.433 [95% CI: 1.168, 10.091]). These results showed an increased risk of dengue hospitalization and severe dengue in seronegative subjects 2-8 years at enrolment in the pool of CYD14+CYD23/57 and also in CYD14.

For subjects aged 2 - 5 years at enrolment and classified as seronegative, the HR/RR of dengue hospitalization pooled across studies (CYD14 + CYD23/57) was > 2. The HRs/RRs were consistent and statistically significant across different methods that ranged from 2.087 (anti-NS1 M13 Threshold 20) to 3.70 (TMLE M13). In CYD14, the HR/RR ranged from 2.21 (TMLE M0) to 3.478 (anti-NS1 Threshold 9) and was statistically significant for all the methods used.

The relative risks of dengue hospitalization occurring after M0 in subjects aged 2-5 years at enrolment and classified as dengue seronegative by PRNT at M0 (MI) was 2.293 (1.157, 4.544) (Source: Table 9.120) for CYD vs. Placebo subjects. . These results showed an increased risk of dengue hospitalization in

seronegative subjects 2 to 5 years at enrolment in the pool of CYD14+CYD23/57. In contrast, HR estimates for severe dengue in seronegative subjects aged 2 to 5 years were similar to what was observed for the entire age subgroup (seronegative subjects 2-8 years).

The CYD14 VE estimate against VCD cases in seronegative subjects 2-8 years was non statistically significant with values of 8.2% for the NS1 Th 9 analysis (M13-M25) and 18.7% for the MI analysis (M0-M25).

2b) Estimate of Risk and Efficacy in Seropositive Individuals 2-8 Years

The risk of dengue hospitalization, the risk of severe dengue, and vaccine efficacy against symptomatic VCD in subjects 2-8 years at enrolment and classified as seropositive by PRNT50 at M0 (imputed/predicted, MI analysis) or by NS1 at M13 (threshold 9) are presented for all studies in Figure 26.

Figure 26: Estimates of risk of hospitalized and severe dengue and efficacy against symptomatic VCD in seropositive subjects 2-8 years.

Serostatus classification Time period	CYD group n	CYD group N	Control group n	Control group N	Subjects 2-8 years at enrollment and classified as seropositive	p-value
					Risk of dengue hospitalization - CYD14+CYD23/57 (HR and 95% CI)	
MI M0 onwards	93.6	313.2	89.8	156.4	0.504 (0.331 – 0.767)	0.002
NS1 Th 9 M13 onwards	75	300	65	135	0.526 (0.358 – 0.774); Favourable / Unfavourable	0.001
					Risk of severe dengue (IDMC) - CYD14+CYD23/57 (HR and 95% CI)	
MI M0 onwards	23.9	313.2	20	156.4	0.578 (0.257 – 1.304)	0.183
NS1 Th 9 M13 onwards	21	300	17	135	0.556 (0.286 – 1.08); Favourable / Unfavourable	0.083
					Vaccine efficacy (VE) against symptomatic VCD - CYD14 (95% CI)	
MI M0- M25	88.5	217.3	104.3	109.5	Unfavourable / Favourable; 0.595 (0.307 – 0.764)	0.002
NS1 Th 9 M13-M25	30	209	42	91	0.698 (0.492 – 0.821)	<0.001

n: number of subjects fulfilling the item listed
N: total number of subjects selected in sub-cohort
NS1 analysis: subjects with VCD cases before M13 are excluded from the analyses
MI analysis: n and N are average numbers from 10 iterations of multiple imputations
Study group classified as treated (Subjects classified as CYD Dengue Vaccine Group if received at least 1 injection of CYD dengue vaccine)

The HR against dengue hospitalization in seropositive subjects 2-8 years at enrolment was <1, and was statistically significant for both methods. These results show a decreased risk of dengue hospitalization in seropositive subjects 2-8 years at enrolment in the pool of CYD14+CYD23/57.

The HR against severe dengue in seropositive subjects <2-8 years at enrolment was <1, for both methods. Although not statistically significant, these results showed a trend towards a decreased risk of severe dengue in seropositive subjects 2-8 years at enrolment in the pool of CYD14+CYD23/57.

Regarding VE, the CYD14 VE estimate against VCD cases (until M25) in seropositive subjects 2-8 years was statistically significant with values of 59.5% for the MI analysis and 69.8% for the NS1 Th 9 analysis.

Long-term data

The Applicant submitted the following data:

- CYD14 and CYD15 HP/SEP safety and efficacy data up to Y3 (SEP: surveillance expansion Phase)
- Pooled safety data (CYD14+CYD15+CYD23/57) up to Y3 HP/SEP. CYD57 was already completed in 2016.
- Pooled efficacy data (CYD14+CYD15) during the surveillance expansion phase (SEP).
- Individual preliminary data on severe VCD during the incomplete Y4 HP/SEP of CYD14 and CYD15.

These data represent interim results, and VE during the entire SEP will be fully described when CYD14 and CYD15 are completed and fully analysed. Of note, VE against VCD cases during the SEP corresponds mainly to data during the Y3 HP/SEP, as only few subjects could be considered in the analysis during the Y2 HP/SEP (2 subjects in CYD14, and 2680 subjects [12.8%] in CYD15 were considered in the Full Analysis Set for SEP). Moreover, caution is required when comparing VE data during the SEP to VE data during the Active Phase due to different epidemiological patterns during the 2 periods (e.g. different serotypes circulation, variable dengue incidence).

The VE against symptomatic VCD due to any of the 4 serotypes during the SEP for CYD14 and CYD15 is presented by age group in Table 22 below.

Table 22: CYD14 &CYD15 – VE against symptomatic VCD due to any serotype during the SEP (Y2 HP/SEP and Y3 HP/SEP) by age group – Full Analysis Set for SEP

		CYD Dengue Vaccine Group (N=6321)				Control Group (N=3135)				Vaccine Efficacy	
	Age group	Cases	Person-years at risk	Density incidence (95% CI)	n Episodes	Cases	Person-years at risk	Density incidence (95% CI)	n Episodes	%	(95% CI)
CYD14	All subjects	97	4959	2.0 (1.6; 2.4)	97	66	2448	2.7 (2.1; 3.4)	66	27.4	(-0.8; 47.5)
	9 to 14 years	30	2422	1.2 (0.8; 1.8)	30	26	1203	2.2 (1.4; 3.2)	26	42.7	(-0.9; 67.2)
	2 to 8 years	67	2537	2.6 (2.1; 3.3)	67	40	1246	3.2 (2.3; 4.3)	40	17.8	(-24.9; 45.2)
	2 to 5 years	36	1099	3.3 (2.3; 4.5)	36	20	543.3	3.7 (2.3; 5.6)	20	11.0	(-62.2; 49.9)
CYD15	All Subjects	32	8275	0.4 (0.3; 0.5)	32	24	4076	0.6 (0.4; 0.9)	24	34.3	(-16.5; 62.5)

Cases: number of subjects with at least one symptomatic VCD episode during the considered period.
Density incidence: data are cases per 100 person-years at risk.
n Episodes: number of symptomatic VCD episodes in the considered period.

In the pooled CYD14+CYD15 data, VE against all symptomatic VCD cases during the SEP was 29.3% (95% CI: 7.5; 46.0) in all subjects and 38.7% (95% CI: 11.1; 57.8) in the subjects aged 9 to 16 years, with a lower bound of the CI above 0 in both cases. It is noteworthy that the dengue incidence in the control group was lower in CYD15 (0.6%) than in CYD14 (2.7% in all subjects and 2.2% in the 9 to 14 years).

Of note these LTFU data do not distinguish between seropositive and seronegative subjects.

In CYD14, the point estimate for the RR for hospitalised Dengue is lower in Year 3 HP/SEP compared to Year 2 HP/SEP, indicating that the risk for hospitalised Dengue is declining over time, except in the youngest age group of 2-5 year old children, where the risk further increased towards a point estimate of approx. 2. The upper limit of the 95%CI comprises 1 in all age categories, indicating that a higher risk compared to the Control group is plausible. The Dengue annual incidence rate has been stable over Year 2 and Year 3 HP/SEP in both vaccine and Control group, i.e. 0.8%. In Year 3 SEP, there were 48 cases of hospitalised Dengue in the vaccine group compared to 28 cases in the Control group.

In CYD15 (9-16 YOA), the point estimate for the RR for hospitalised Dengue is higher in Year 3 HP/SEP compared to Year 2 HP/SEP, but there were few cases and the annual Dengue incidence rate in CYD15

was low, i.e. about 0.2-0.3 in the Control group and this further diminished to <0.1% in Year 3 HP/SEP for both groups. In Year 3 SEP, there were 7 cases of hospitalised Dengue in the vaccine group compared to 4 cases in the Control group. When pooling all cases over the Hospital Phase/SEP, both the RR point estimate and the 95%CI upper limit are <1. This pattern is similar over the Active Phase and over the Entire Study indicating a decreased risk in the overall 9-16 year old children 5 years post-dose 1.

In the pooled efficacy analysis, the previous conclusions by age group are confirmed:

- RR<1 in the 9-16 year children, indicating a decreased risk for hospitalised dengue by vaccination
- RR>1 in the 2-5 year children, indicating an increased risk for hospitalised dengue by vaccination (safety signal)
- RR <1 in the 2-8 year children, but 95%CI upper limit >1.

In CYD14, the point estimate for the RR for severe Dengue is lower in Year 3 HP/SEP compared to Year 2 HP/SEP, indicating that the risk for hospitalised Dengue is declining over time, except in the youngest age group of 2-5 year old children, where the risk increased towards a point estimate of approx. 2. The upper limit of the 95%CI comprises 1 in all age categories, indicating that a higher risk compared to the Control group is plausible. The Dengue annual incidence rate has been stable over Year 2 and Year 3 HP/SEP in both vaccine and Control group, i.e. about 0.2%. In Year 3 SEP, there were 38 cases of severe Dengue in the vaccine group compared to 16 cases in the Control group.

In CYD15 (9-16 YOA), the point estimate for the RR for severe Dengue could not be calculated for Year 3 HP/SEP, but there were 4 cases in the vaccine group compared to 0 cases in the Control group. When pooling all cases over the Hospital Phase/SEP, the RR point estimate is 0.898 and the 95%CI upper limit is >1. However, over the Active Phase and over the Entire Study the RR is <1 indicating a decreased risk in the overall 9-16 year old children 5 years post-dose 1.

In the pooled efficacy analysis, the previous conclusions by age group are confirmed:

- RR<1 in the 9-16 year children, indicating a decreased risk for severe dengue by vaccination
- RR>1 in the 2-5 year children, indicating an increased risk for severe dengue by vaccination (safety signal)
- RR <1 in the 2-8 year children, but 95%CI upper limit >1.

Based on exploratory analysis during a period of 5 years after the first injection, in subjects 9 and above dengue seropositive at baseline, the vaccine efficacy (1-Hazard Ratio) was estimated at 79% (95% CI: 69; 86) for hospitalized VCD and 84% (95% CI: 63; 93) for severe VCD. The same analysis could not be done for symptomatic VCD because data over the entire period of 5 years was not available because of the break between active phase and SEP.

Data from year 3 and 4 of study CYD57

This is a brief summary of CYD57 study data from Y3 and Y4 of the study, which were submitted during the procedure.

The RR fluctuated over time regardless of the age group. In children ≥9 years of age (9-11 years), the RR against hospitalized VCD cases was: 0.307 in Y1, 0.307 in Y2, 0.171 in Y3, and 1.120 in Y4. During the 4 years of the Hospital Surveillance the RR against hospitalized VCD cases was of 0.511 (95% CI: 0.25; 1.04) indicating a trend toward a decreased risk of hospitalized VCD cases in subjects who received the CYD dengue vaccine compared to the Control Group. In subjects ≥9 years of age, the pooled RR during the entire study was 0.421 (95% CI: 0.23; 0.75).

During Y3 and Y4 HP, only 2 cases of hospitalized severe VCD were reported each year. Overall, from D0 of CYD23 study to the end of CYD57, there were 10 hospitalized severe VCD cases in the Dengue Group and 5 in the Control Group (RR of 1.003 [95% CI: 0.31; 3.74]). The very limited number of hospitalized severe VCD cases does not allow the analysis of results by age group.

The long-term systematic follow-up of SAEs has not shown evidence of excess of any specific SAE in the vaccinated group as compared to the Control Group.

Summary of immunogenicity data across trials

The immunogenicity of the final formulation of the CYD dengue vaccine administered 6 months apart was assessed in 16 clinical studies: 2 large-scale pivotal efficacy studies CYD14 and CYD15 and 14 supportive studies, i.e. 9 Phase II (CYD08, CYD12, CYD13, CYD22, CYD24, CYD28, CYD30, CYD47 and CYD51), 1 Phase IIb (CYD23) and 4 Phase III (CYD17, CYD29, CYD32 and CYD33). All these 16 CT are consistent in terms of the general study design, vaccine formulation and schedule, and they all used the dengue PRNT50 immunoassay for the assessment of the neutralizing titres against dengue parental strains. Thus, the approach of performing an Integrated immunogenicity analysis was considered appropriate.

The main objective was to provide an overview of the humoral immune response against each and any dengue serotype induced by the CYD dengue vaccine 28 days after each of the 3 vaccine injections administered 6 months apart according to age, region and dengue immune status at baseline. In the two pivotal trials CYD14 and CYD15, reactogenicity and immunogenicity were not assessed in all subjects, but only in a subset of the participants. This subset included 2000 subjects from several countries in each of the two trials, which represent about 20% (CYD14) and 10% (CYD15) of those recruited in the trials. Dengue immune status at baseline was defined as follows: i) Immune: subjects with quantified neutralizing Ab against at least one dengue serotype in the baseline sample, ii) Non-immune: subjects without quantified neutralizing Ab against any of the 4 dengue serotypes in the baseline sample.

Most of the subjects included in CTs whose immunogenicity was analysed were from endemic regions (n=4907) and most of them were children 6 to 11 years (n= 1833) and adolescents 12 to 17 years (n= 1510). From endemic regions, only data from 294 subjects from 18-45 years were available. For adult subjects (18 to 60 years) in total 873 subjects from non-endemic regions were included in the studies.

The percentage of dengue immune subjects at baseline varied widely in different trials both in the Asia Pacific (AP) and Latin America (LatAm) endemic regions. In the AP endemic region ranged from 26.7% (CYD28) to 86.5% (CYD47), being 68.1% in the pivotal trial CYD14. In the LAtAM region varied from 3.9% (CYD29) to 80.7% (in pivotal trial CYD15). Moreover, the Dengue and FV immune status at baseline varied across the age groups, both in the AP and in LatAM endemic region. In fact, in trial CYD14 the percentage of subjects dengue seropositive at baseline were: 53.9% (2-5 y); 71.8 (6-11 y); 80.0% (adolescents), and in CYD15 were 76.9% (6-11 y); and 84.3% (adolescents).

An increase in GMTs was observed for each of the 4 serotypes after 3 injections of the CYD dengue vaccine. Generally, a trend toward lower post dose 3 (PD3) GMTs was observed against serotype 1 compared to the 3 other serotypes. Nonetheless, PD3 GMTs varied widely across studies depending on known factors such as region, age group, and baseline dengue immune status.

Immunogenicity Results According to Region:

Overall immune responses varied across regions. No data are available with the final vaccination schedule of the CYD dengue vaccine in adolescents and children in non-endemic region. Similarly, no data are available for adults in LatAm endemic region. PD3 GMTs remained lower in non-endemic regions compared to endemic regions. For example, for adults from non-endemic regions, and for serotype 1, the

PD3 GMTS were 24 (CYD12), 18 (CYD 17) and 14 (CYD51), whereas from subjects from endemic regions the GMTs were 695 (CYD22), 48 (CYD28) and 461 (CYD 47). The same pattern is observed for the other three serotypes. In addition, the percentage of adults who were seropositive against all 4 serotypes PD3 was lower in non-endemic region (50.0% to 62.9%) than in endemic regions (56.7% to 100%). These data indicate lower immunogenicity of the vaccine in from non-endemic regions. In LatAm endemic regions, a trend to higher PD3 GMTs was observed for each of the 4 serotypes compared to AP endemic countries, despite limited differences in baseline titers between the 2 regions. This trend was more limited than the one observed between non-endemic and endemic regions.

Immunogenicity Results According to Age:

A comparison across the different age groups was only feasible in endemic regions where the 5 age groups, i.e. adults, adolescents, children 6-11 years, children 2-5 years, and infants and toddlers, were included in the studies.

Baseline and PD3 GMTs against each serotype were usually higher in adults and adolescents compared to children 6 to 11 years, 2 to 5 years and infants and toddlers. For example, in trial CYD14, the baseline GMTs for these groups for serotype 1 were: 93.1 (adolescents), 42.6 (6-11 years), and 15.7 (2-5 years). Similarly, the PD3 GMTs remained lower in infants and toddlers, children 2 to 5 years and 6 to 11 years compared to adolescents and adults. For example in CYD14, for serotype 1, the PD3 GMTs were 305 (adolescents), 149 (6-11 y), and 109 (2-5 years).

In agreement with these GMT data, the percentage of subjects who were seropositive against all 4 serotypes PD3 tended to be lower in children 2 to 5 years and 6 to 11 years compared to infants and toddlers, adolescents and adults.

Overall, there was a relationship between baseline and PD3 levels of GMTs against each serotype: higher PD3 GMTs were observed in subjects with higher GMTs at baseline.

Immunogenicity Results According to Dengue and Other FVs immune status

The general overview of the immune response showed that there is a relationship between dengue immune status at baseline and the immune response after the third injection of the CYD dengue vaccine. Baseline and post dose 3 GMTs in subjects 9 to 16 years of age in CYD14 and CYD15 are shown in Table 23, with data stratified according to dengue serostatus at baseline and per serotype. Results from the 2 pivotal studies illustrate that higher PD3 GMTs were observed in baseline dengue immune subjects compared to baseline dengue non-immune subjects. These differences appear to be statically significant since the 95%CI of these estimates does not overlap in any of the four strains.

Table 23: Immunogenicity for subjects 9 to 16 years of age in CYD14 and CYD15 from endemic areas according to Dengue serostatus at baseline

			Serotype 1		Serotype 2		Serotype 3		Serotype 4	
Study	**Dengue serostatus at baseline**	**N**	**Pre-injection 1 GMT (95%CI)**	**Post-injection 3 GMT (95%CI)**	**Pre-injection 1 GMT (95%CI)**	**Post-injection 3 GMT (95%CI)**	**Pre-injection 1 GMT (95%CI)**	**Post-injection 3 GMT (95%CI)**	**Pre-injection 1 GMT (95%CI)**	**Post-injection 3 GMT (95%CI)**
CYD14	**positive**	485	167 (138; 202)	437 (373; 511)	319 (274; 373)	793 (704; 892)	160 (135; 190)	443 (387; 507)	83.8 (72.0; 97.6)	272 (245; 302)
	negative	128	5.00 (NC)	33.3 (25.7; 43.0)	5.00 (NC)	114 (88.8; 146)	5.00 (NC)	57.9 (45.0; 74.4)	5.00 (NC)	63.0 (49.9;79.6)
CYD15	**positive**	1048	278 (247; 313)	703 (634; 781)	306 (277; 338)	860 (796; 930)	261 (235; 289)	762 (699; 830)	73.3 (66.6; 80.7)	306 (286; 328)
	negative	251	5.00 (NC)	35.3 (29.8; 41.9)	5.00 (NC)	105 (89.3; 125)	5.00 (NC)	93.6 (80.3; 109)	5.00 (NC)	89.5 (76.1; 105)

N: number of subjects with available antibody titre for the relevant endpoint
Seropositive subjects are subjects with titres above or equal to LLOQ (1/dil) against at least one dengue serotype at baseline
NC: Not computed

The percentage of vaccinated subjects that became seropositive to all four serotypes was lower in the baseline dengue non-immune population than in those immune at baseline. Importantly, this observation is maintained in all trials and for all age groups. In study CYD14, in adolescents only 63.3% of the non-immune subjects at baseline seroconverted to all four dengue serotypes, whereas 97.5 % of those immune at baseline seroconverted; for CYD15, these figures were 69.9% vs. 98.2%.

The impact of previous exposure to other FV infection (which can be the result of either prior vaccination against other FV and/or prior infection by other FVs) was also analysed. FV analysis was limited to the following: JE in Asian studies or dengue and YF in LatAm studies. The impact of previous exposure to FV is described in the subset of subjects seronegative to dengue at baseline, in order to describe the possible effect of pre-existing neutralizing Ab to YF or JE on the immune response to dengue vaccination. The choice of this subset is based on the observation of the lack of specificity of the YF PRNT assay as well as various FV cross-reactive responses described in the literature. Post-injection 3 GMTs against each serotype in dengue seronegative/FV seropositive adults, adolescents, children, and infants and toddlers were similar to those in dengue seronegative/FV seronegative adults, adolescents, children, and infants and toddlers. Similar trends were observed in both endemic regions. However as the number of evaluated subjects is limited, definitive conclusions could not be drawn.

Immunogenicity data in adults

Of the 24 studies which are part of the dossier:

- 4 Phase I trials, 5 Phase II trials, and 1 phase III trial (lot-to-lot consistency) enrolled adults in non-endemic areas.
- Only 1 Phase I trial and 3 Phase II trials enrolled adults in endemic areas (CYD22 in Vietnam, CYD28 in Singapore, CYD47 in India; all evaluating the final formulation). All were done in Asia.

Data from 1167 adults are available for immunogenicity with the final formulation:

- 873 adults 18-60 years old from non-endemic regions.
- 294 adults 18-45 years old from endemic regions (vaccinated group), including 148 subjects from the CYD28, 126 from the CYD47 and 20 from the CYD22. Available data on the persistence of

antibodies up to 4 years in endemic countries mainly derive from the CYD28 Study (see also section 2.5.5).

Baseline and post-dose 3 GMTs in subjects 18 to 45 years of age are shown in Table 24, with the data stratified according to the dengue serostatus at baseline and per serotype.

Table 24: Immunogenicity for subjects 18 to 45 years of age from endemic areas according to Dengue serostatus at baseline

			Serotype 1		Serotype 2		Serotype 3		Serotype 4	
Study	**Dengue serostatus at baseline**	**N**	**Pre-inj 1 GMT (95%CI)**	**Post-inj 3 GMT (95%CI)**	**Pre-inj 1 GMT (95%CI)**	**Post-inj 3 GMT (95%CI)**	**Pre-inj 1 GMT (95%CI)**	**Post-inj 3 GMT (95%CI)**	**Pre-inj 1 GMT (95%CI)**	**Post-inj 3 GMT (95%CI)**
CYD22	**positive**	19	408 (205; 810)	785 (379; 1626)	437 (240; 797)	937 (586; 1499)	192 (117; 313)	482 (357; 651)	86.5 (41.2; 182)	387 (253; 591)
	negative	1	5.00	89.0	5.00	95.0	5.00	47.0	5.00	219
CYD28	**positive**	66	59.8 (36.8;97.4)	235 (135; 409)	67.1 (40.9; 110)	236 (144; 387)	48.4 (32.9;71.0)	239 (166; 342)	22.1 (14.7;33.4)	211 (155; 287)
	negative	74	5.00 (NC)	14.6 (11.3; 18.8)	5.00 (NC)	26.4 (19.2; 36.3)	5.00 (NC)	39.1 (30.5;50.1)	5.00 (NC)	79.5 (55.9; 113)
CYD47	**positive**	109	324 (236; 445)	688 (524; 901)	363 (269; 490)	644 (509; 814)	394 (299; 519)	961 (763;1211)	80.7 (61.3; 106)	413 (331; 516)
	negative	17	5.00 (NC)	46.1 (23.7; 89.7)	5.00 (NC)	94.3 (36.6; 242)	5.00 (NC)	123 (69.3; 218)	5.00 (NC)	103 (74.5; 141)

N: number of subjects with available antibody titre for the relevant endpoint
Seropositive subjects are subjects with titres above or equal to LLOQ (1/dil) against at least one dengue serotype at baseline
NC: Not computed; Pre-inj: pre-injection

The baseline seropositivity levels were higher in adults than in other age categories. Seropositivity rates at baseline was lower in the CYD28 as compared to the two other studies for adults (18-45 years) (baseline serotype-specific seropositivity ranged from 80% to 90% in the CYD22 and 78% to 87% in the CYD47; in the CYD28, the frequency of subjects immune at baseline against at least one serotype was 47%).

Overall vaccination increased the antibody titres to each of the 4 serotypes regardless of pre-vaccination titres. GMTs after vaccination varied depending on dengue serostatus at baseline (higher titres in subjects seropositive at baseline), serotype (responses tended to be lower to serotype 1), age (titres tend to increase with age) and region (linked to endemicity).

For long term immunogenicity data see section 2.5.5.

Extrapolation of VE to Subjects Not Included in the Efficacy Studies

Pivotal efficacy data were obtained in AP in subjects aged 2 to 14 years (CYD14) and in LatAm in subjects aged 9 to 16 years (CYD15). In order to evaluate the potential benefit of vaccinating populations in which no efficacy data are available, it is important to evaluate if VE could be expected in this population.

The extrapolation of the results observed in the Phase III efficacy trials populations to individuals 18 to 45 years living in endemic areas was based on immunological data from 2 supportive studies (CYD22 in Viet Nam and CYD47 in India) that were conducted in endemic regions in adult subjects aged 18 to 45 years (see previous subsection). Immunogenicity results were compared to those obtained in the 2 pivotal efficacy studies CYD14 and CYD15, through:

- An analysis of the PD3 GMTs, and of the ratio of GMTs, ratio below 1 indicating a higher PD3 immune response in CYD22 and CYD47 as compared with the reference pivotal efficacy study population.

- The distribution of baseline and 28-days post-injection 3 titres was descriptively compared through RCDCs.

It was noted that of the 20 subjects included in trial CYD22, only one was dengue seronegative at baseline. Similarly, for study CYD47 only 17 of the 126 subjects were seronegative at baseline.

The immunogenicity data from these studies suggest that PD3 GMTs against each serotype in adults are generally comparable to those observed in CYD14 and CYD15 populations where efficacy was demonstrated. Given that the 2 pivotal efficacy studies showed an association between levels of PD3 titres and probability of the disease (see Section Ancillary analyses), it is reasonable to expect a similar level of protection following CYD dengue vaccination in individuals aged 17 to 45 years from endemic regions compared to the VE observed in the CYD14 and CYD15 studies. In addition, as compared to the efficacy trials, in CYD22 which included also children and adolescents subjects, a similar relationship was observed between the age and the PD3 titres: overall the older the subjects, the higher the PD3 GMTs were.

In addition using statistical methods to estimate the VE based on immunogenicity data, imputed VE in adults in endemic countries ranged from 68% to 84%.

CYD28 data in adults have not been used for the extrapolation strategy because the extrapolation strategy is applicable to people living in countries with high dengue seroprevalence and is not applicable to countries of low endemicity such as Singapore. The post-dose-3 humoral responses seen in study CYD28 were lower than the GMTs in children and adolescents in the pivotal studies.

Data in Adults 46-60 Years

Immunogenicity data in adults 46 to 60 years are only available from non-endemic regions (CYD17 study conducted in Australia) and showed that PD3 GMTs are similar in subjects 46 to 60 years of age (N= 241) compared to subjects 18 to 45 years (N= 414). This age group is at the moment not included in the MAA.

2.5.4. Ancillary analyses

Immunogenicity Data in Relation to Efficacy Data

The Applicant presented analysis to investigate the relationship between antibody titres measured by PRNT50 and PRNT90 and the risk of dengue disease and vaccine efficacy based on the overall study CYD14/15 population. Briefly, to evaluate the relationship between the occurrence of the disease and the level of Log10 antibody (Ab) titre after the third injection, analyses were performed for symptomatic VCD cases of each and any serotypes explained by the PD3 titres (from the homologous titre first and then considering the 4 PD3 titres) as well as other covariates and interactions. The logistic and multivariate models used were designed to detect the association between antibody titres and risk of dengue disease and not to predict protection level based on the estimates. In addition the Applicant investigated the association between VE and the level of Ab titres based on PRNT titres after the third injection of the CYD dengue vaccine.

In summary a link between PD3 PRNT titres and VE has been shown with both PRNT assays: the higher the titre, the lower the risk of VCD. There is some evidence to suggest that titres measured by PRNT90 may be more discriminatory compared to PRNT50 in predicting risk of dengue disease. It was not possible to stablish a threshold titre that conferred protection.

Moreover, the Applicant provided the immunogenicity data (including PRNT50 and PRNT90 data) stratified by serostatus comparing dengue breakthrough cases vs. non cases in the vaccine group. The Applicant re-analysed immunogenicity data with both PRNT50 and PRNT90. These data are consistent

with those summarised above, i.e. GMTs tend to be higher in non-cases compared to cases for each serotype with both assays, although some variability was observed. It was also noted that some breakthrough cases had high neutralizing antibody titres following vaccination.

Concomitant vaccination

Immune response to the 4 DENV serotypes have been investigated in toddlers by comparative immunogenicity studies (CYD08, CYD29, CYD33) of CYD vaccine given alone or concomitantly with other live vaccines such as measles/mumps/rubella (MMR) vaccine or YF vaccine, or concomitantly with DTaP-Hib-IPV vaccine or pneumococcal conjugate vaccine. One study was conducted in adults from non-endemic regions on concomitant administration of the first injection of the CYD dengue vaccine with the yellow fever vaccine (CYD51 study: 120 subjects 18 to 45 years of age).

Available results so far did not raise any safety concerns and did not show any impact on the immune response to the concomitant vaccines or to the CYD dengue vaccines. However no enough data are available in the indicated population to issue recommendation for use.

Safety and immunogenicity of co-administration of CYD dengue vaccine with other vaccines is currently being evaluated in 3 studies: CYD66 assesses the co-administration with Tdap (Adacel) in 9-60 YOA in the Philippines, CYD67 assesses the co-administration with HPV vaccine (Gardasil) in 9-13 YOA in Malaysia, and CYD71 assesses the co-administration with HPV vaccine (Cervarix) in 9-14 YOA in Mexico.

2.5.5. Supportive studies

Persistence of Immunogenicity

Ab persistence data up to 2 years after vaccination were assessed essentially from CYD14, CYD15 and CYD23 studies. Limited supportive data up to 4 years after vaccination in CYD22 and CYD28, and up to 5 years after vaccination in the early development study CYD05 (Phase I; a schedule 0, 3/4, 12 months was used in this study) was also assessed. Preliminary long term follow data up to 4 years post-vaccination from the pivotal studies were presented by the Applicant during the procedure.

The Ab persistence is mainly presented through 2 parameters:

1. GMTs at baseline, after the third injection (PD3), and each year after the third injection (Y1, Y2, Y3, Y4 and Y5) to reflect the level of Ab for each serotype;
2. GMTRs against each serotype:
    - Yearly/PD3 to reflect the magnitude of the decrease for each serotype, i.e. the lower the GMTR, the higher the decrease is.
    - Yearly/baseline to reflect the level of remaining Abs for each serotype, i.e. GMTR>1 means that PD3 GMTs are higher than baseline levels.

The 2-year Ab persistence (GMTs) in the pivotal studies is presented in Table 25 by age group and overall, regardless of the dengue immune status at baseline.

Table 25: CYD14 and CYD15 - Summary of persistence of GMTs of dengue Abs against each serotype, according to age group – FASI

			Serotype 1				Serotype 2			
Age group	**Region**	**Study**	**N**	**PD3 GM (M) (95% CI)**	**1 year FUP GM (M) (95% CI)**	**2 year FUP GM (M) (95% CI)**	**N**	**PD3 GM (M) (95% CI)**	**1 year FUP GM (M) (95% CI)**	**2 year FUP GM (M) (95% CI)**
12-16YOA	**Endemic AP**	**CYD14**	400	305 (396) (249; 372)	247 (387) (198; 308)	217 (388) (173; 271)	400	592 (396) (506; 692)	425 (390) (357; 505)	324 (387) (272; 386)
	Endemic LatAm	**CYD15**	658	466 (651) (399; 545)	343 (639) (289; 409)	254 (613) (214; 301)	658	684 (651) (605; 772)	472 (639) (410; 542)	415 (613) (364; 475)
6-11YOA	**Endemic AP**	**CYD14**	468	149 (466) (126; 176)	106 (459) (86.7; 129)	82.3 (461) (67.3; 101)	468	321 (464) (280; 368)	198 (460) (168; 234)	137 (459) (115; 162)
	Endemic LatAm	**CYD15**	643	333 (640) (285; 390)	204 (621) (169; 245)	172 (608) (143; 205)	643	480 (640) (429; 538)	290 (624) (252; 333)	277 (609) (240; 320)
2-5YOA	**Endemic AP**	**CYD14**	455	109 (454) (93.5; 126)	49.2 (443) (40.6; 59.5)	45.3 (436) (37.2; 55.2)	455	252 (454) (222; 287)	95.2 (447) (81.2; 112)	83.2 (437) (69.8; 99.1)
			Serotype 3				**Serotype 4**			
12-16YOA	**Endemic AP**	**CYD14**	400	309 (396) 261; 367)	350 (391) (290; 422)	224 (378) (185; 272)	400	213 (396) (185; 245)	146 (389) (127; 169)	132 (381) (115; 153)
	Endemic LatAm	**CYD15**	658	554 (651) (488; 628)	356 (640) (309; 411)	348 (611) (304; 397)	658	277 (651) (252; 305)	214 (640) (193; 237)	157 (613) (141; 174)
6-11YOA	**Endemic AP**	**CYD14**	468	222 (464) (190; 259)	208 (459) (176; 247)	123 (452) (103; 147)	468	153 (465) (137; 172)	86.9 (459) (75.5; 100)	65.8 (453) (56.8; 76.2)
	Endemic LatAm	**CYD15**	643	466 (640) (412; 527)	238 (624) (204; 277)	263 (607) (227; 304)	643	210 (640) (191; 231)	141 (624) (126; 159)	121 (609) (109; 135)
2-5YOA	**Endemic AP**	**CYD14**	455	136 (454) (119; 155)	95.2 (447) (81.3; 112)	64.4 (424) (53.8; 77.1)	455	110 (454) (98.6; 122)	52.5 (444) (45.6; 60.4)	42.7 (433) (36.8; 49.5)

Table 26: CYD14 and CYD15 - Summary of persistence of GMTs of dengue Abs against each serotype in the overall population – FASI

	Serotype 1				**Serotype 2**			
Study	**N**	**PD3 GM (M) (95% CI)**	**1 year FUP GM (M) (95% CI)**	**2 year FUP GM (M) (95% CI)**	**N**	**PD3 GM (M) (95% CI)**	**1 year FUP GM (M) (95% CI)**	**2 year FUP GM (M) (95% CI)**
CYD14	615	255 (611) (217; 299)	211 (597) (176; 251)	178 (600) (149; 213)	615	530 (609) (469; 600)	372 (601) (323; 428)	278 (596) (241; 321)
CYD15	1301	395 (1291) (353; 441)	266 (1261) (234; 302)	209 (1222) (185; 237)	1301	574 (1291) (528; 624)	371 (1264) (336; 409)	339 (1223) (307; 374)
	Serotype 3				**Serotype 4**			
CYD14	615	289 (609) (253; 331)	312 (601) (269; 361)	203 (585) (174; 237)	615	201 (610) (181; 223)	132 (602) (118; 148)	114 (587) (101; 128)
CYD15	1301	508 (1291) (465; 555)	292 (1265) (263; 325)	303 (1219) (274; 334)	1301	241 (1291) (226; 258)	174 (1265) (161; 188)	138 (1223) (128; 149)

During the first 2 years of follow-up in the 2 pivotal studies, GMTs for each serotype decreased in all age groups in both studies. The decrease of GMTs against all 4 serotypes from Year 1 to Year 2 tended to be lower than between PD3 and Year 1 in all age groups. Despite this decrease, GMTs for each serotype remained at higher levels than those observed at baseline in all age groups.

These observations are further illustrated by the RCDCs presented in the main population included in CYD14, i.e. children 6 to 11 years, and in CYD15, i.e. adolescents 12 to 16 years.

Data from the pivotal trials up to 4 years post dose 3 was presented, showing a trend to a stabilization of the GMTs against all 4 serotypes. Overall, GMTs for each serotype up to 4 years remained similar to or higher than baseline levels in all age groups of the claimed population in the indication.

Impact of age on Ab persistence

When considering each age group, regardless of the dengue immune status at baseline, a trend toward lower GMTRs Y2/PD3 was observed for each serotype in younger children as compared with adolescents. Therefore, the younger the subject, the higher the decrease of GMTs observed.

However overall, GMTs 2 years after vaccination for each serotype remained higher than at baseline for all age groups, with GMTRs Y2/baseline above 1.

Impact of dengue immune status at baseline on Ab persistence

When considering the dengue immune status of subjects at baseline, regardless of age, a trend toward lower GMTRs Y2/PD3 was observed for each serotype in baseline non-immune subjects compared to baseline immune subjects. Therefore, baseline dengue non-immune subjects tended to have a higher decrease of GMTs for each serotype than baseline dengue immune subjects, in all age groups.

However overall, GMTs 2 years after vaccination for each serotype remained higher than at baseline for both baseline dengue immune and non-immune subjects, with GMTRs Y2/baseline above 1.

In conclusion, in all age groups:

- A decrease in the level of Abs (GMTs) against all 4 serotypes was observed 1-2 years after the third injection, followed by a trend for a lower decrease in subsequent years.
- The decrease in the level of Abs was variable according to age and dengue immune status of subjects at baseline. The decrease was higher in younger subjects and in those who were seronegative at baseline.
- Long-term GMTs for each serotype remained overall higher than baseline values.
- The same conclusions were observed in the claimed population in the indication, i.e. subjects from 9 through 45 years in endemic regions.

Study CYD17

Title: Lot-to-Lot Consistency and Bridging Study of a Tetravalent Dengue Vaccine in Healthy Adults in Australia.

Methodology: This was a multi-centre, observer-blind, randomized, placebo-controlled, Phase III study of 4 lots of CYD dengue vaccine in 715 healthy adult subjects aged 18 to 60 years in Australia. Each subject was to receive a 3-dose primary series of injections (at month 0, 6, and 12), followed by a 6-month safety follow-up.

Main Objective: Lot Consistency. To demonstrate that three different Phase III lots of CYD dengue vaccine induce an equivalent immune response in terms of post-Dose 3 geometric mean titres (GMTs) against the four parental serotypes.

Results and conclusion: Result Equivalence of the GMTs 28 days after the third injection was statistically demonstrated in the PP Analysis Set for each pair of lots for each serotype (11/12 comparisons) except for serotype 2 (Lot 1-Lot 2) where the upper limit of the 95% CI was higher than the pre-defined limit. The fact that one of the 12 statistical comparisons was not achieved is not considered relevant.

Study CYD29

Title: Immunogenicity and Safety of Yellow Fever Vaccine (Stamaril) Administered Concomitantly with Tetravalent Dengue Vaccine in Healthy Toddlers at 12-13 Months of Age in Colombia and Peru.

Methodology: Randomized, observer-blind (for the group allocation), multi-centre, Phase III trial in 792 healthy toddlers in Colombia and Peru administered an injection of Stamaril vaccine concomitantly with the first dose of CYD dengue vaccine or placebo at 12-13 months of age.

Main Objective: To demonstrate the non-inferiority of the immune response against YF in FV non-immune subjects at baseline receiving one dose of Stamaril vaccine administered concomitantly with the first dose of CYD dengue vaccine compared to subjects receiving one dose of Stamaril vaccine concomitantly with placebo.

Results and conclusion: The co-administration of Stamaril with CYD dengue vaccine results in a good YF Ab response, with no clinically relevant impact on the safety profile of Stamaril. Similarly, the co-administration of Stamaril with CYD dengue vaccine did not impact the immunogenicity and safety for CYD dengue vaccine in toddlers.

Study CYD33

Title: Immunogenicity and Safety of a Booster Injection of DTaP-IPV//Hib (Pentaxim) Administered Concomitantly with Tetravalent Dengue Vaccine in Healthy Toddlers Aged 15 to 18 Months in Mexico.

Methodology: Randomized, observer-blind (for the second dose of tetravalent dengue vaccine), open-label (for the first and third doses of tetravalent dengue vaccine), multi-centre, Phase III trial in 624 healthy toddlers in Mexico administered a booster injection of Pentaxim vaccine concomitantly with the second dose of tetravalent dengue vaccine at 15 to 18 months of age.

Main Objective: To demonstrate the non-inferiority of the antibody (Ab) response against all antigens (diphtheria, tetanus, pertussis, polio and Hib) in subjects receiving one booster dose of Pentaxim vaccine administered concomitantly with the second dose of CYD dengue vaccine compared to subjects receiving one booster dose of Pentaxim vaccine administered concomitantly with placebo.

Results and conclusion: The co-administration of Pentaxim vaccine with CYD dengue vaccine results comply with the non-inferiority in all antigens. Pentaxim vaccine elicited an acceptable seroprotection / booster response (as defined for each antigen) in both Group 1 and Group 2.

Study CYD32

This study was a Phase III study to evaluate safety and immunogenicity of the vaccine in a paediatric population in Malaysia (2-11 years of age). Results were consistent with the other studies and the overall immunogenicity findings of the pivotal studies.

Study CYD28

Title: Immunogenicity and Large-Scale Safety of Tetravalent Dengue Vaccine in Healthy Subjects Aged 2 to 45 Years in Singapore.

The CYD28 trial is one of the three late Phase II studies evaluating the safety and immunogenicity of the final formulation of the CYD dengue vaccine in endemic countries. The study enrolled 1198 healthy subjects aged 2 to 45 years in Singapore, including 695 adults of whom 521 received CYD. This is one of the few trials that enrolled adults from endemic countries and that provided data on the persistence of antibodies up to 4 years in endemic countries.

Results: In this trial, 47% of the adults were immune to dengue at baseline (at least one serotype), and 21% were immune to all 4 dengue serotypes. The seroprevalence level is much lower in adolescents and children (14% and 20% respectively).

CYD induced high humoral responses in all age categories. The seropositivity and GMTs levels increased at each dose, with high levels achieved post-dose 3 in all age categories and for all serotypes. Humoral responses (seropositivity levels and GMTs) were higher for serotype 3 and 4 than for serotypes 1 and 2. For serotype 1, seropositivity levels post dose 3 tended to be lower in adolescents and adults.

Seropositivity levels and GMTs decreased markedly at year-1 after dose 3 as compared to 28 days post dose 3 (by approximately 10% to 60% depending on serotype and age), and continued to decrease thereafter. The levels remained globally higher than at baseline except for serotype 1 for which persistence is low and the level at 4-year close to the baseline level. Ab persistence is better for serotype 4. At 4-year follow-up, seropositivity rates against at least 1 serotype remained high compared to baseline. However, seropositivity rates against 3 or 4 serotypes greatly decrease and were close to the baseline levels.

During the vaccination phase (based on post-dose 3 data), immunogenicity in terms of seropositivity and GMTs tended to be lower in adults and adolescents than children. However, ab persistence was much better for adults (the decrease of seropositivity rates between post-dose 3 and 1-year follow-up was 23%-54% in children; 19%-62% in adolescents; 7%-36% in adults). This translated in higher level of ab at 1, 2, 3 and 4-years for adults compared to children.

The immunogenicity of CYD in terms of GMTs was much lower in seronegative subjects as compared to subjects who were immune at baseline, whatever the post-vaccination timepoints and age category. For GMTs, this difference was more pronounced in adults and in adolescents than in children. High percentages of subjects non-immune at baseline became seropositive after 3 injections (89.6% to 96.5% in children, 62.9% to 89.7% in adolescents, 59.4% to 87.5% in adults. The persistence of immunity at 4-year in terms of the percentage of subjects seropositive against at least 3 serotypes was low in seronegative subjects whatever their age.

2.5.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The Clinical Development Program (CDP) for Dengvaxia followed the WHO guidelines available at the time of initiating the Clinical Trials. Moreover, EMA, FDA and several European national regulatory authorities advised on the CDP. All trials were performed in several countries from endemic areas, and the design of the studies and the endpoints were in agreement with WHO recommendations. For the purpose of this application, an endemic region was defined as a region where the disease has been continuously present in the native population with documented outbreaks or epidemics. A non-endemic region was defined as a region where the disease has been generally absent in the native population with no documented outbreaks or epidemics.

Due to lack of an immunological correlate of protection for dengue, it was necessary to demonstrate the clinical efficacy of this vaccine. One supportive Phase IIb proof-of-concept efficacy study CYD23 was performed in children aged 4 to 11 years in Thailand, followed by two pivotal large-scale Phase III efficacy studies, CYD14 in children aged 2 to 14 years in Asia Pacific (AP), and CYD15, in children aged 9 to 16 years in Latin America (LatAm).

The two pivotal trials were adequately performed in two geographical areas (Latin America and Asia Pacific, including 5 countries per region) where Dengue is endemic. Although the percentage of

Caucasians (the main ethnic group in Europe) included in the trials was low, there is no reason to believe that the vaccine will behave differently in different ethnic groups, and thus it is considered that the immunogenicity and efficacy data obtained from these trials can be extrapolated to the EU population.

Subjects included in CYD14 and CYD15 studies were respectively 2 to 14 and 9 to 16 years of age, which is considered the critical age for more severe dengue disease. Phase IIb study CYD23 included subjects aged 4 to 11 years. Following an excess of hospitalized dengue cases vs. control in children 2-5YOA in studies CYD14 and CYD23/57, the Applicant decided to pursue an indication from 9 YOA. No efficacy data are available for subjects older than 16 years. In all three trials subjects were randomized in a 2 to 1 ratio to receive either three injections of the vaccine administered 6 months apart or three placebo injections (saline solution). No other vaccine against Dengue is currently available. Administration of the vaccine was observer-blinded, i.e. neither the vaccinee nor the Investigator in charge of safety evaluation did know which product had been injected.

In CYD14 and CYD15, immunogenicity was assessed only in a subset of 2,000 subjects from several countries in each of the two trials (full analysis set for immunogenicity, FASI), which represented about 20% (CYD14) and 10% (CYD15) of those recruited in the trials. This approach was in accordance with WHO recommendations given the challenges of testing samples from all subjects, however in view of the results of the clinical trials, this size of the immunogenicity subset turned out to be an important limitation. Of note, based on the inclusion criteria, all subjects irrespective of serostatus at baseline (i.e. subjects with/without quantified neutralizing antibodies against any of the 4 dengue serotype) were included in the trials.

The efficacy studies were designed in 2 phases to detect dengue cases:

- The Active Phase, from the day of the first injection until 13 months after the third injection (25 months), to actively detect symptomatic VCD cases, regardless of the severity. This phase included the primary endpoint observation period from 28 days after the third injection up to the end of the Active Phase (PD3 period).
- The Hospital Phase, in which dengue screening occurred in febrile subjects who required hospitalization. Hospitalized and severe hospitalized dengue cases were collected for 4 years from the end of the Active Phase, i.e. up to 5 years after the third injection (CYD23 subjects were followed-up through the CYD57 follow-up study). Active surveillance was not initially foreseen in the protocol for the long term data collection during the hospital phase, and it turned out to be of importance.

Efficacy data were collected only during the Active Phase, and this set of data is completed. Hospital Phase contributed to the assessment of the long-term follow-up for safety of the CYD dengue vaccine and since this Phase was still ongoing at the time of this application, the final analyses will be submitted post-authorisation.

The Primary Objective in the three trials was to determine the efficacy of the CYD dengue vaccine in preventing the occurrence of symptomatic VCD cases, regardless of the severity, over the PD3 period due to any of the 4 serotypes in children aged 2 to 16 years at inclusion. The Secondary Objectives during the Active Phase were to describe VE in preventing the occurrence of symptomatic VCD cases due to each of the 4 serotypes during the PD3 period, and to describe VE in preventing the occurrence of symptomatic VCD cases during the whole Active Phase period (25 months) due to: i) any of the 4 serotypes, and ii) each of the 4 serotypes. Primary and secondary endpoints were in agreement with WHO guidelines and they are considered appropriate. Case definitions for VCD, severe VCD and hospitalised VCD were considered adequate and in agreement with WHO recommendations.

The most relevant populations in relation to the efficacy analysis are: Per Protocol Analysis Set for Efficacy (PPSE), Full Analysis Set for Efficacy (FASE) and Modified Full Analysis Set for Efficacy (mFASE). The PPSE and the mFASE were used for the analysis of VE from 28 days post-Dose 3 to the end of the Active Phase (the so called post-dose 3 period, PD3), but whilst the former included only subjects who had no protocol deviations, the later included all subjects redgardless of per-protocol criteria. The FASE included all subjects who received at least one injection, and was used to assess efficacy from 28 days after the injection up to the end of the Active Phase or from the first injection to the end of the Active Phase. Compliance was good in all efficacy studies, with more than 95% of subjects receiving 3 injections of either the CYD dengue vaccine or the control. The Per-Protocol Set for efficacy population was near 98.0% and 90.0% in CYD14 and CYD15, respectively, therefore the PPSE and mFASE populations were almost equivalent in terms of interpretation of results.

The efficacy data covered the individual estimates for the 2 pivotal efficacy studies CYD14 and CYD15, an Integrated Efficacy analyses (IEA) describing the integrated estimate from the meta-analysis of CYD14 + CYD15, the individual estimate for proof-of-concept study CYD23 and the integrated estimate from the meta-analysis on CYD14+CYD15+CYD23 as supportive data.

The use of a validated plaque reduction neutralization test (PRNT) to determine the immunogenicity of the vaccine was considered adequate although it has limitations in terms of specificity, due to cross-reactivity with other flaviviruses. Hence misclassification of subjects (false positives) cannot be excluded. PRNT90 is more specific than PRNT50 with regard to cross-reacting antibodies against flavivirus. At present these neutralisation assays are considered among the most specific assays for determining dengue serostatus at baseline.

Based on the FASI population, the proportions of dengue immune subjects at baseline were high for all efficacy studies. The proportion of baseline dengue immune subjects in CYD15 (~80%) is higher than in CYD14 (~68%). This result reflects the older age group recruited in CYD15 and the different regional epidemiology in LatAm versus Asia. Considering the different immunogenicity and efficacy of the vaccine depending on the dengue immune status at baseline, the percentage of subjects seronegative at baseline has an impact on the overall VE determined in different geographical locations.

During the conduct of the Active Phase, the dengue incidence in the control group was higher than the incidence expected from epidemiological data and that was the data used for the sample size calculation. This contributes to narrowing the 95%CI of the VE.

The main hypothesis postulated to explain the initial observation of an imbalance in hospitalised dengue cases in the youngest vaccinees in CYD14 (2-5year-olds) during the first year of the hospital phase is the increased risk in CYD vaccinees who have not been exposed to dengue prior to being vaccinated. In analyses of vaccine efficacy and risk in CYD14 and CYD15, serostatus (as a surrogate of prior dengue exposure) was identified as an important covariate. To circumvent the limited precision with which the impact of serostatus on vaccine performance could be estimated due to the limited number of subjects that had pre-vaccination sample collected (the immunogenicity subset was 10-20% of the total pivotal trials population), the Applicant undertook a supplemental study to impute the baseline serostatus in a larger dataset based on dengue anti-NS1 IgG ELISA assay and PRNT50 assay (the so called supplemental NS1 extension study). The supplemental NS1 extension case-cohort study was thus performed post-hoc for expanding the existing data on both VE and potential risk of dengue hospitalization and/or severe dengue according to baseline serostatus in the CYD dengue vaccine efficacy trials, by using the blood sample that was collected in all subjects at M13. Overall, although more prone to biases and mistaken inference than a design using the overall cohort, the NS1 study design was considered an acceptable alternative in this context. The selections of the sub-cohort and of the cases were appropriate, i.e. representative of the actual cohort that gave rise to the cases. A limitation of the current analysis is the

absence of long-term efficacy data against symptomatic VCD which will be analysed at the end of the Surveillance Expansion Phase. The principal analysis determined risk of dengue hospitalization/severe dengue and VE against symptomatic VCD based on PRNT50 at baseline to determine serostatus. PRNT50 baseline serostatus was either measured (for subjects in the immunogenicity subset) or predicted -in subjects with missing baseline values- by 2 separate methods using available M13 dengue anti-NS1 values and other covariates (e.g. age vaccine group, country). In addition, complementary assessments used serostatus classification in the expanded case-cohort study based on M13 anti-NS1 readouts. Subjects were classified as dengue seropositive or seronegative based on two alternative cut-off thresholds of 9 EU/mL and 20 EU/mL. The dengue anti-NS1 IgG ELISA assay at a threshold of 9 EU/ml was considered sensitive and appropriate for the identification of naïve dengue individuals for the purpose of this analysis. The choice of measured/predicted PRNT50 at baseline as a primary surrogate for prior dengue infection (anti-NS1 at M13 as secondary surrogate) is endorsed, given the potential of differential misclassification with the post-vaccination anti-NS1 assay due to influence of the CYD vaccination on the read-out (cross-reactivity with YF NS1).

Efficacy data and additional analyses

Efficacy data in children

1. Primary objective

The efficacy of the CYD dengue vaccine in CYD14 and CYD15 in the PPSE showed an overall reduction of respectively 56.5% and 60.8% in VCD cases due to any serotype during PD3 period in a population aged 2 to 16 years living in dengue endemic regions and having received the full immunization schedule. In both trials the primary objective was met since the lower bound of the 95% CI of VE was >25%, however vaccine efficacy is considered modest. Results were confirmed in the mFASE population. In CYD23 (a phase II study), VE was 30.2%, with 95%CI including 0, so VE could not be demonstrated in this trial. The result was primarily driven by the fact that approximately 60% of the VCD cases were due to serotype 2, against which VE was not demonstrated. These results were driven by lower efficacy in younger children, i.e. 2-5YOA.

However when considering only subjects of 9-16YOA in line with the proposed indication, VE against symptomatic VCD was higher (mFASE, PD3 period): 69.4% in CYD14 (95% CI 52.2; 80.6), 61.3% in CYD15 (95% CI 52.8; 68.2), 70.1% in CYD23 (95% CI 9.3; 91.1). The pooled analysis showed a VE of 62.8% in CYD14+CYD15 (95% CI 55.7; 68.8) and 63.0% in CYD15+CYD15+CYD23 (95% CI 56.1; 68.9).

2. Secondary Objectives

Over the whole 2 years of Active Phase period (FASE population), again VE against VCD cases due to any serotype in 2 to 16 YOA was demonstrated in the 2 pivotal efficacy studies and VE estimates were consistent with those observed during the PD3 period (PPSE). In this population (FASE), VE in trial CYD23 was lower than in trials CYD14 and CYD15, but the lower bound of the 95%CI was above 0. In subject 9-16YOA, VE against symptomatic VCD during the whole active phase (FASE) was 67.8% in CYD14 (95% CI 57.7; 75.6), 64.7% in CYD15 (95% CI 58.7; 69.8) and 43.3% in CYD23 (95% CI -18.9; 72.7). The pooled analysis in the active phase showed a VE against VCD of 65.6% (95% CI 60.7; 69.9) for CYD14+CYD15 and of 64.9% (95% CI 60.0; 69.2) for CYD15+CYD15+CYD23.

The efficacy of the CYD dengue vaccine against VCD cases due to each of the 4 serotypes was demonstrated in the 2 pivotal efficacy studies during the whole Active Phase period. Nonetheless, VE varied across serotypes, with a lower VE for serotypes 1 and 2 as compared to serotypes 3 and 4. In the pooled analysis of CYD14 + CYD15 the VE estimates were 54.7% (95%CI: 45.4; 62.3), 43.0% (95% CI: 29.4; 53.9), 71.6% (95%CI: 63.0; 78.3) and 76.9% (95% CI: 69.5; 82.6) for serotype 1 to 4,

respectively. Importantly, the 95%CI of the VE estimates for serotypes 3 and 4 do not overlap with the 95%CI for serotypes 1 and 2. This observation indicates that VE of the vaccine is statistically higher for serotypes 3 and 4 than for serotypes 1 and 2, and this may impact on VE in different settings depending on the serotypes circulating in those settings. Results followed a similar trend for the population 9-16YOA, although efficacy was higher (e.g. 47.1% against serotype 2 see Table 20).

VE estimates against VCD cases due to any serotypes in each country showed important variability, ranging from 31.3% (95% CI: 1.3; 51.9) in Mexico (CYD15) to 79.0% (95% CI: 52.3; 91.5) in Malaysia (CYD14). The main factors that explain the lower VE observed in Mexico are: i) mainly serotypes 1 and 2 were circulating in this country and ii) the very low baseline rates of dengue seropositivity, which is a known covariate of VE. In Brazil VE was 77.5% (95% CI: 66.5; 85.1), a result mainly due to high baseline rates of dengue seropositivity combined with predominant circulation of serotype 4.

3. Other objectives

VE against clinically severe VCD cases (according to IDMC, Independent Data Monitoring Committee) due to any serotype was demonstrated in the 2 pivotal efficacy studies during the whole Active Phase period. In the pooled analysis of CYD14+CYD15, VE against clinically severe VCD was 79.1% (95%CI: 60.0-89.00) in the overall study population and 93.2% (95% CI 77.3; 98.0) in subjects 9-16YOA. In the pooled analysis of CYD14+CYD15, VE against clinically severe dengue was shown for each of the 4 serotypes.

Overall the CYD14+CYD15 pooled data show that the CYD dengue vaccine reduced the occurrence of any hospitalized VCD cases during the whole Active Phase in children aged 9 to 16 years (VE of 80.8% (95% CI 70.1; 87.7). A similar trend of efficacy level against hospitalized VCD cases was observed for the 4 serotypes. The VE estimates were in line with those obtained for VCD and severe cases. VE against DHF meeting WHO criteria was 92.9% (95% CI 76.1; 97.9) in the Active Phase for 9-16year-olds.

4. Efficacy according to covariate

- Age: overall, the CYD dengue vaccine reduced the occurrence of any VCD cases during the whole Active Phase period in the different age groups. In subjects 2-8YOA VE against symptomatic VCD cases during PD3 due to any of the 4 serotypes was 40.5% (95% CI: 22.7; 54.2) (pooled CYD14+CYD23). In this age group, VE against symptomatic, severe and hospitalized VCD during the whole active phase due to any serotype (pooled CYD14+CYD23), was respectively 42.2% (95% CI: 30.6; 51.8), 45.1% (95% CI: 29.3; 76.7), 47.6% (95% CI: 23.7; 64.0). VE in youngest subjects (2-5 years) was the lowest (pooled CYD14+CYD23: 35.2%, 95% CI: 16.1-49.9) with a trend toward increasing efficacy with age. It is however difficult to dissociate the impact of age from baseline dengue status as these 2 variables are interlinked. VE appears to be maintained from 8 to 16 years of age.
- Dengue serostatus: VE against symptomatic VCD cases during the whole Active Phase due to any of the 4 serotypes was demonstrated in subjects Dengue seropositive at baseline. In the pooled analysis of CYD14+CYD15, VE estimate was 78.2% (95% CI: 65.4; 86.3), similar to VE estimates in the two individual trials. VE against VCD cases was lower when both Dengue seropositive and seronegative subjects at baseline were considered (VE=60.3; 95%CI: 55.7; 64.5). Importantly the 95%CI of the two estimates do not overlap which it is indicative that VE in the whole population and VE in seropositive subjects at baseline are different. In agreement with these observations, in the pooled analysis of CYD14+CYD15 in baseline dengue seronegative subjects VE estimate against symptomatic VCD cases during the whole Active Phase due to any of the 4 serotypes was 38.1% (95% CI: -3.4; 62.9), and similar VE were seen in the 2 individual studies. This result is inconclusive

since the 95%CI spans 0 but the trend is towards lower efficacy in seronegatives, which may explain the overall modest efficacy observed for the primary endpoint in the entire study population.

- Other Flavivirus (FV): A potential effect of prior exposure to either JE or YF on vaccine efficacy could not be clearly established nor ruled out, due to the small number of cases in which this effect could be studied.

5. Supplementary efficacy analyses by dengue baseline serostatus

➢ *Post-hoc exploratory analysis of efficacy in the immunogenicity subset*

The efficacy analysis by serostatus (PRNT50 at baseline) was done in the FASE or mFASE using the data from subjects included in the immunogenicity subset. One criterion to be part of the FASI was to have a blood sample drawn after injection, which was not a necessary condition for the efficacy analysis. Subjects were counted in the efficacy analysis as long as they have a baseline status and even if no sample were taken after injection.

VE against symptomatic VCD in subjects 9 to 16 years of age, seropositive at baseline from the immunosubset of studies CYD14+ CYD15 +CYD23 was 79.4% (95% CI 58.4; 89.8) in the PD3 period and 81.9% (95% CI: 67.2 ; 90.0) for the whole active phase. In these subjects, one clinically severe VCD case and one WHO DHF VCD case was reported during the whole active phase in the control group in each individual study (CYD14 and CYD15) versus none in the vaccine group. Four hospitalized VCD cases in CYD14 and two hospitalized VCD cases in CYD15 were reported in the control group versus none in the vaccine group. These data are inconclusive due to the low number of cases in the immunogenicity subset. However, vaccine efficacy (1- Hazard Ratio), obtained from an exploratory analysis (pooled CYD14 +CYD15+CYD23) during the active phase, is estimated at 89.2% (95% CI: 78.5; 94.6) for hospitalized VCD and 95.3% (95% CI: 68.9; 99.3) for severe VCD.

In baseline dengue seronegative subjects aged 9-16 years VE in the pooled CYD14+CYD15 was 52.5% (95% CI: 5.9; 76.1).

Exploratory analyses stratifying seropositive individuals by the number of serotypes to which they were seropositive prior to vaccination (by PRNT50 and PRNT90, FASI population) provide reassurance about vaccine protection: in individuals who are seropositive to only one serotype (surrogates of "monotypic immune status"), who are at highest risk of serious/severe dengue if unvaccinated, a risk reduction of ~77% against hospitalized dengue was estimated, and in those who are seropositive to more than one serotype (surrogate of "multi-typic immune status") a risk reduction of ~71% against hospitalized dengue was estimated.

➢ *NS1 supplemental analysis based on dengue anti-NS1IgG ELISA and PRNT50 assays*

An important limitation of the present data submission is that the dengue serostatus at baseline was only determined in a small subset of study participants, i.e. in ~4000 subjects (10-20% of all CT subjects) of which nearly 75% were seropositive for at least one dengue serotype at baseline. PRNT50-based efficacy analyses by serostatus were thus of limited statistical precision, and although allowed to estimate VE in seropositive subjects, they were inconclusive regarding VE in seronegative subjects and inconclusive regarding impact of serostatus according to age. The supplemental NS1 extension studies used a case-cohort design and mainly aimed at evaluating vaccine efficacy and risk of dengue-associated outcomes in subjects assigned to CYD dengue vaccine as compared to control groups for subjects from efficacy trials (CYD23/57, CYD14, and CYD15) retrospectively classified as dengue naïve at baseline.

Different methodologies were used to define baseline serostatus and overall there was consistency in the estimates of risk and efficacy across the different methodologies. The results of this extension study clearly confirmed the predominant influence of baseline serostatus on VE regardless of age.

In the subjects aged 9 to 16 years, these investigations showed a clear and long-term benefit of CYD vaccine in the seropositive population with protection against symptomatic dengue up to M25 and long-term protection against hospitalized and severe dengue. In the seronegative population, a potential limited short-term benefit against symptomatic VCD is offset by an increased risk of hospitalized and severe dengue. Estimates from the long-term analysis suggest that the onset of increased risk was mainly during the 3rd year following the first injection. The pooled analysis of CYD14+CYD15 VE estimate against VCD cases in seronegative subjects 9-16 YOA over the whole active phase was 18% and 38% depending on the method used, but with a lower bound of the CI below 0 (p-value 0.054).

The CYD14 VE estimate against VCD cases in seronegative subjects 2-8 years was non statistically significant with a value of 18.7% for the MI analysis (M0-M25, active phase period).

When this risk of hospitalized and severe dengue in the seronegative population was analysed by age strata, an imbalance was observed in both the 9-16 and 2-8 year old population, albeit the estimates of risk were only statistically significant for several of the methods in the 2-8 year old age group and for the outcome of hospitalized dengue in this age group. Further stratification by age revealed a statistically significant increased risk of hospitalized dengue in the seronegative 2 - 5 year old age stratum (HR > 2) only. In CYD14, where the original observation of an increased risk in subjects aged 2 - 5 years (regardless of serostatus) was detected, the NS1 supplemental analysis narrows the scope of this risk to only seronegative subjects. In the subset of CYD14, the risk of hospitalized dengue was similar in seronegative subjects aged 6 - 8 years and subjects aged 9 - 11 years (HR > 1; not statistically significant), but the risk was not found in seronegative subjects aged 12 – 14 years (HR < 1; not statistically significant). In CYD15, although the magnitude of the risk decreased in the seronegative 12 - 16 year-old age group compared to the seronegative 9 – 11 year-old age group, the HR was > 1 in both age groups.

Over a period of 5 years since the first injection, in subjects 9 to 16 years of age with no previous dengue infection, the risk of severe dengue increased by 2.43 fold (95% CI: 0.47; 12.56) in vaccinees as compared to control subjects. This increased risk at an individual level would translate into 5 additional hospitalized and 2 additional severe dengue cases over a 5 year period for every 1000 seronegative subjects vaccinated, for settings with incidence of dengue consistent with the clinical trials.

In contrast, in seropositive subjects the analysis of pooled studies showed a decreased risk of dengue hospitalization in vaccinated versus unvaccinated seropositive subjects. All of the estimated HRs/RRs were < 1 and statistically significant in the 2-8 years and 9-16 years old subjects. This statistically significant decreased risk was also observed in each individual study and consistent across methods, studies, and for all 4 serotypes. Data were consistent with severe dengue. For every 1000 dengue seropositive subjects 9-16YOA vaccinated, 15 hospitalized and 4 severe dengue cases are estimated that could be prevented, for settings with incidence of dengue consistent with the clinical trials. Although these prediction methods all relied on using measured anti-NS1 data and other variables as predictors, and varying assumptions, the consistency of the patterns of the estimates of risk and efficacy across different methodologies utilized strengthens the robustness of the findings and conclusions.

The NS1 analyses confirmed the conclusions of the analyses on the immunogenicity subset, i.e. CYD vaccine showed high efficacy in seropositive subjects in every age category, whereas in seronegatives efficacy is measurable but inconclusive. For individuals who have experienced at least one dengue virus

infection, CYD vaccine seem to constitute a booster of pre-existing immunity which protects against infections with new heterologous serotypes, regardless of age.

A possible effect of age on vaccine performance, particularly evident for the outcome of hospitalized dengue in study CYD14, could not be ruled out. As age is known to be associated with dengue exposure, it remains unclear whether these findings of a risk associated with CYD dengue vaccine reflect the effect of dengue exposure.

Immunogenicity data in children and adults

The immunogenicity of the final formulation and schedule of the CYD dengue vaccine was assessed in 16 clinical studies. The clinical development program demonstrated strong and persistent humoral immune response induced by CYD.

An increase in GMTs was observed for each of the 4 serotypes after 3 injections of the CYD dengue vaccine. The vaccine appeared to be much less immunogenic in subjects dengue seronegative at baseline as compared to those seropositive (to at least one dengue serotype) at baseline, in terms of both GMT titres and percentage of subjects that seroconverted to all four dengue serotypes. Generally, a trend toward lower post dose 3 (PD3) GMTs was observed against serotype 1 compared to the 3 other serotypes, however PD3 GMTs varied widely across studies depending on serotype, region, age group, and baseline dengue immune status.

Limited immunogenicity data was generated in subjects 18-45 YOA in this application in non-endemic and endemic regions. Three studies (CYD22, CYD28, CYD47) were conducted with the final formulation and final schedule in an adult population in endemic regions. For the age group 46 to 60 years, only one trial CYD17 (N=241) was conducted in a non-endemic region. The baseline seropositivity levels ranged from 47% to 90% in CYD22, CYD28 and CYD47 studies, and were higher in adults than in other age categories. So the results could be confounded by the different baseline immune status of the different age groups. Immunogenicity data from the CYD22 and CYD47 studies (respectively N=20 and N=126 aged 18-45 years in the CYD vaccine group) suggested that PD3 GMTs against each serotype in adults were generally comparable to PD3 GMTs observed in children in studies CYD14 and CYD15. In contrast, in study CYD28, (521 subjects 18 to 45 years in the vaccine group) PD3 humoral responses are lower in adults as compared to children and adolescents enrolled in the same study; however local endemicity is also different in this region.

Based on efficacy data from the pivotal trials, the Applicant analysed the relationship between the occurrence of VCD cases and the level of neutralizing antibody titre 28 days after the third injection in order to identify a CoR (i.e. an association between antibody titres and risk of dengue disease) and a CoP. A link between PD3 titres and VE has been shown with both PRNT assays: the higher the titre, the higher the VE. However it was not possible to establish a threshold antibody titre that conferred protection and antibody titres alone may not fully explain protection against disease.

Evaluation of CMI responses (by ICS and CBA) was also assessed in some studies in adolescents and adults in endemic and non-endemic regions (studies CYD04, CYD10, CYD11 and CYD28). However, as the role of these responses in protection from DENV infection is largely unknown, these data are hard to benchmark against clinical data.

Efficacy in adults

In the absence of efficacy data in adults and of a correlate of protection for dengue, the Applicant performed complementary analyses to support the bridging between immunogenicity data and efficacy. Immunogenicity results obtained in adults were compared to those obtained in the 2 pivotal efficacy studies CYD14 and CYD15.

The immunogenicity data from two studies conducted in adults aged 18 to 45 years in endemic regions (CYD22 and CYD47) suggested that PD3 GMTs against each serotype in adults were generally comparable to PD3 GMTs observed in CYD14 and CYD15 populations where efficacy was demonstrated. Given that the 2 pivotal efficacy studies showed an association between levels of PD3 titres and probability of the disease and a similar relationship across age groups for high titres, it is reasonable to expect a similar level of protection following CYD dengue vaccination in individuals aged 18 to 45 years from endemic regions compared to the VE observed in the CYD14 and CYD15 studies. It is important that confirmatory data on adults is collected from post licensure effectiveness studies in endemic countries, based on vaccination implementation by countries in this age range.

Limitations with the dose schedule and booster

The decision to select a three-dose vaccination schedule was based mainly on data in seronegatives, and justified by the necessity to overcome poor immunogenicity in the seronegatives. Therefore when all the subjects included in the trials are considered -and especially when seropositive subjects are considered- the increase in GMT titres comparing PD3 to PD2 data is limited.Since the study objective was to assess VE after a 3-injection regimen, the Applicant performed exploratory analyses on all subjects on the efficacy data between injections in CYD14 and CYD15 (between injection 1 and injection 2, between injection 2 and injection 3, between injection 3 and 6 months after injection 3 and between 6 months after injection 3 and the end of the Active Phase). For the pooled data analysis of CYD14 + CYD15, VE estimates against VCD cases due to any serotype between each injection and up to the end of the Active Phase were all similar, approximately 60%, showing that VE is relatively stable after the first injection up to the end of the Active Phase both in the 2-16 and the 9-16 year-olds. Therefore it remains unclear whether protective immunity in seropositive individuals could be achieved with less than three doses considering that all evidence including long term is based on a 3 dose schedule. Morevoer it is not possible to recommend a different posology at this stage. Post-authorisation studies may provide more information, e.g. immunogenicity and safety of one-dose and two-dose vaccination schedule will be investigated in study CYD65.

The need for additional booster doses remains to be elucidated. Study CYD65 will investigate a booster dose one or two years after the last injection. Two other studies, CYD63 (Singapore) and CYD64 (Latin America) are underway to assess the effect of a booster dose of the CYD dengue vaccine 4 to 5 years after the third dose (PD3) of the primary series administered in previous studies (CYD28 and CYD13/30, respectively). In both CYD63 and CYD64, a booster dose has already been administered to participants and data from interim analysis were presented. Although these studies are not powered to assess the need of a booster dose according to the dengue serostatus of the subjects at baseline (i.e. before the primary series), the booster significantly increased titres in seronegatives and restored the level of titres induced after the primary series in seropositive subjects. These data provide some support that a booster vaccination is reasonably likely to confer clinical benefit however the final analyses of trials CYD63 and CYD64 are needed before any conclusions can be made. Moreover, the impact of the booster may differ by country depending on local endemicity.

LTFU data

The pooled LTFU data indicate that the risk for hospitalised and/or severe dengue is below 1during the HP/SEP in subjects 9-16YOA vs. placebo (0.535 [95% CI: 0.38; 0.75]. Overall in the Entire study the RR for hospitalized VCDs and severe VCDs is decreased (i.e. is below 1) for 9-16YOA and 2-8YOA. During the period of 5 years after the first injection (Y3 of HP), in subjects 9 and above seropoisitive at baseline, vaccine efficacy (1-Hazard Ratio) (obtained from exploratory analysis) is estimated at 79% (95% CI: 69; 86) for hospitalized VCD and 84% (95% CI: 63; 93) for severe VCD. A corresponding analysis on VCD could not be done because data over the entire period of 5 years was not available because of the break

between active phase and SEP. Data from years 4 to 6 of the study (i.e. after 1st injection, which correspond to year 2 to 4 of the SEP) showed a VE against VCD in subjects 9 to 16 of 38.7% (95% CI: 11.1; 57.8). However these data are preliminary and do not distinguish between seronegative and seropositive at baseline. The Applicant commits to provide the final CSRs of CYD14 and CYD15 post-authorisation as soon as available.

Based on antibody (Ab) data available up to 4 years after the third injection in CYD14 and CYD15, and exploratory efficacy data, it could be anticipated that protective efficacy may be maintained albeit at lower levels than that observed in the first 3 years of the study.

The risk of severe dengue disease due to waning protection against dengue disease over time is proposed as an important potential risk in the RMP and will therefore be followed up post-authorisation.

Additional expert consultation

The CHMP Scientific Advisory Group on Vaccines was convened during the procedure to address 5 clinical questions raised by the CHMP. The final SAG answers are reported as follows.

1. **Concerning the increased risk of hospitalised/severe dengue that was observed in the pivotal studies:**

   a) **Does the SAG agree that being seronegative to all 4 dengue serotypes constitutes an identified risk factor for severe dengue?**

   b) **Does the SAG have any additional observations from the data on factors predisposing to severe dengue upon vaccination with CYD?**

   c) **Can the SAG comment on the relative risk of hospitalised/severe dengue in subjects seropositive to e.g. one or two serotypes and on the efficacy of the vaccine in the same subjects based on different assays (e.g. PRNT50/90)?**

The SAG unanimously concluded that based on the available evidence negative serostatus at baseline is clearly associated with increased risk of severe dengue following vaccination, at least in the period of follow up in the clinical trials, and specifically for those who were seronegative to all 4 serotypes.

Concerning point 1.b) the SAG discussed by analogy factors that may potentially trigger the severe form of the disease in children (e.g. differences in nutrition, previous immunity, concomitant infections, maturation of the immune system); however, since the mechanism by which disease can be enhanced by vaccination is not established for certain, it is not possible to understand how these factors would translate into increased risk of severe disease. Other potential factors that were discussed include different efficacy by serotype (as efficacy for DENV 3 and 4 is higher than for DENV 1 and 2), which may mask the effect of age seen on vaccine efficacy, the role of immune system maturation in younger children (quality and titres of neutralising antibodies), and antibody decay following vaccination, which may be faster in younger children. It is also plausible that young infants, born to vaccinated mothers, may be at increased risk of severe dengue as would be the case following natural infection of the mothers.

Overall it was not possible for the SAG to identify any other factors different than serostatus that may predispose to severe dengue, mainly because the clinical trials were not designed to provide such information. However it was also noted that the pattern of severity is similar to that seen in seropositive unvaccinated individuals, strongly suggesting a similar mechanism. In addition the available data may not fully exclude that age could be a contributing factor in terms of increased risk of severe/hospitalised dengue.

Concerning point 1.c), the SAG discussed the new data presented by the MAH at the meeting, whereby an efficacy of 77% against symptomatic VCD was seen across studies in subjects seropositive to one single serotype by $PRNT_{90}$. These data were considered consistent with the data previously generated across trials. $PRNT_{90}$ was found to be more discriminatory between monotypic and multitypic seropositivity than $PRNT_{50}$, however neither assay is able to provide an exact picture of the individual's past exposure to dengue in terms of distinguishing subjects infected by 2, 3 or 4 serotypes. In addition the results measured by PRNT are influenced by the time elapsed from infection. Whilst the NS1 data represent reasonably good evidence that individuals infected by at least one serotype can largely benefit from vaccination, it is not possible to further dissect these data to discriminate the cumulative effect of subsequent serotype infection on vaccine efficacy. Currently no test can reliably discriminate seropositivity to one, two, three or four serotypes. In any case, it was importantly stressed that the number of cases in the respective subgroups of the CYD14/15 trials is too small to be able to draw any conclusion in these post-hoc analyses as the studies were not powered for such subgroup analyses. It is also not possible to conclude on a link between seropositivity by a specific serotype and risk of severe dengue based on the available data. In any case, the most relevant subgroups would be those with no or only one previous infection.

2. **What is the view of the SAG on the Applicant's proposal to limit the use of the vaccine to subjects who are seropositive for at least one dengue type by applying a minimum age of 9 years across some or all endemic areas as a reliable way to minimize the risk of severe dengue and to maximize the benefit in vaccinated subjects residing in EU endemic areas?**

Based on the current evidence, the SAG concluded that even in areas where dengue is endemic, an age cut-off alone is not considered sufficient to minimise the risk of severe dengue seen in seronegatives and that, within the age range proposed by the MAH for the indication (9-45 YOA), the vaccine should only be used in subjects who have a documented exposure to dengue virus by serology. When deciding this, the SAG took into account the fact that endemicity and seroprevalence are highly variable and changeable over time for dengue virus, and that even during outbreaks both remain moderate in EU territories.

Although acknowledging this is outside of the proposed indication, the SAG also discussed the data recently generated in children <9YOA by multiple imputation based on the NS1 assay, which indicate that dengue seropositive 6-8 year-olds (~65% VE and 0.4 hazard ratio for hospitalised cases) or potentially even 2-5 year-olds may also benefit from vaccination. However given thethe need for a diagnostic test that would have a high positive predictive value if used in an age range with very low seroprevalence even in areas of high endemicity, the concerns due to lack of maturation of the immune system and the lack of quantitative predictions of benefits as well as relative and absolute risk of Dengvaxia use in <9YOA, it is not possible to make any conclusive remark at this stage. Overall it was felt that more information is required in order to be able to clarify uncertainties and define the impact of the vaccine in children <9YOA. It was also noted that the data are currently insufficient to define whether the excess of severe cases among seronegative vaccinees could be later offset by a reduction in the medium term. In this respect, the MAA should put efforts in following the clinical trials cohorts in the long term (see also answer to Q5).

3. **Does the SAG consider that limiting the use of the vaccine by positive serostatus at baseline could be a sufficient strategy to minimize the risk of severe dengue occurring in vaccinated subjects residing in EU endemic areas?**

    a) **If so, based on current knowledge, can the SAG comment on the feasibility of using a dengue-specific serology test?**

b) Does the SAG have any alternative or additional suggestions for vaccine usage that might limit the risk of developing severe dengue?

The SAG discussed that there are at least 3 key factors to consider when defining a population that may benefit from Dengvaxia: serostatus at baseline, endemicity and age. It was agreed that limiting the use of the vaccine to seropositive subjects 9-45YOA residing in endemic areas would be the best possible strategy under the current knowledge to limit the risk of developing severe dengue as seen in seronegative subjects. In light of the well-known heterogeneity of dengue epidemiology, a seroprevalence-driven vaccination policy is deemed impractical and not sufficiently reliable regardless of endemicity.

It was also acknowledged that the vaccine is intended for use in European territories which are so far characterised by a lower endemicity than the regions included in the clinical trials. The interaction between dengue endemicity and vaccine performance, including increased risk, is not fully clear yet, so it is not known how different rates of virus circulation over time or potential differences between Asian and Latin-American serotypes may affect vaccine performance in real life settings. The SAG recognised that the use of the vaccine is intended to be for seropositive subjects residing in endemic areas. However the use in selected seropositive travellers frequently visiting endemic regions should not be prohibited.

Furthermore, the SAG was asked to discuss the risk of severe dengue in early infancy in children born to vaccinated women; however the SAG is not aware of any such signal coming from the clinical trial data. It is considered that vaccine-induced antibodies that transfer to the foetus during pregnancy would naturally wane around 3-6 months after birth; hence a potential risk linked to waning antibodies cannot be excluded.

Concerning the assay (point 3.a)), although it should be ultimately up to PHAs to decide which assay to use, it was agreed that a dengue-specific assay should be well characterised, robust and fit for purpose with respect to the endemicity of the EU regions. Different specificity and sensitivity, which will have to be tested in appropriate validation procedures, may indeed be required depending on the endemicity of a particular region (i.e. low seroprevalence/endemicity requires a highly specific test). Currently available neutralising assays, such as $PRNT_{50/90}$, although highly specific, are difficult to perform and would not represent a practical way forward for vaccine implementation. Other assays, such as IgG ELISAs, may be more practical to use, however as mentioned their lower specificity may be problematic in regions with low/moderate endemicity where the risk of false positives is higher, with particular reference to Zika cross-reactivity. In addition it was considered desirable that the performance of a new assay would be linked to the clinical trials data generated with $PRNT_{50}$ in order to allow for appropriate comparison.

Concerning point 3.b), in light of all the limitations mentioned, the SAG could not come up with other recommendations for the CHMP that would help to decrease the risk of severe dengue, apart from testing serostatus pre-vaccination.

4. What is the SAG view on the potential impact of Dengvaxia on the infectivity of Zika and other flaviviruses, and vice-versa impact of such flaviviruses on Dengvaxia protection from Dengue disease?

In vitro and mice studies have been reported showing the potential reciprocal ability of Zika or Dengue cross-reactive antibodies to cause disease enhancement. However this link is not yet fully clarified. Moreover, limited studies in monkeys have not been able to show such interplay between Zika and Dengue and epidemiological data have not yet identified any signal of increased risk of clinical sequelae following Zika infection in Dengue immune individuals Therefore the clinical relevance of such laboratory data is unknown and no conclusions can be made at this stage.

Data from the vaccine studies did not hint to any increased risk of Zika infection following vaccination. The relevance of clinical trial data is however limited because an effect on outcomes of interest (e.g. congenital Zika syndrome) cannot be captured since events are rare. Notwithstanding it is recommended that this aspect be followed up post-authorisation.

5. **Does the SAG have any recommendations to make regarding additional sponsored studies and/or how the abovementioned risks could be monitored in post-approval vaccine effectiveness studies?**

The SAG considered that the best data on vaccine safety and efficacy was generated by the well-designed randomised clinical trials and thus a follow up by enhanced surveillance of the cohorts set up in the trials would be potentially beneficial to gather more information on the long term efficacy of the vaccine, waning of efficacy/immunity and need for and timing of booster doses. It would be especially useful, depending on availability of blood samples from the pivotal trials, if serostatus at baseline is retrospectively identified by NS1 assay testing for the whole cohorts to be able to link serostatus to risk of sever dengue in the long term. The SAG were particularly concerned to learn that follow up of these cohorts in the future was not planned, although having further robust data from these studies is critical for further evaluation of risk/benefit that cannot be readily and robustly obtained post marketing.

The SAG recommended that the final data from the SEP phase of the efficacy trials CYD14/15 should be requested from the company, which may provide further insights into waning efficacy and symptomatic VCD cases.

Additionally it is acknowledged that specific booster studies are planned or ongoing. Investigation of the best posology in seropositive subjects should also be conducted post-approval.

Further information should be obtained retrospectively from the data gathered in the Philippines and Brazil from the implementing vaccination programmes (especially from the cases of deaths); post-marketing surveillance should include occurrence of severe dengue and waning of efficacy. However it is acknowledged that the quality of the data from post-marketing surveillance will be lower due to increased bias than what can be obtained from following up the cohorts of the clinical trials. Also, the quality of the post-marketing data is largely influenced by the country infrastructure and the quality of surveillance, so in this regard Brazil could be viewed as a better option to conduct an effectiveness study. In addition, the feasibility of such studies may be hampered by a low vaccine uptake. Serological testing in these populations is not routine but is strongly desirable to better inform the questions. Collection of data from these populations is encouraged so to attempt a better understating of the protection level and risks associated with baseline serostatus.

Considering the requirement to vaccinate only seropositive subjects at baseline, further data that will be generated post-approval will be expected to have serostatus assessment pre-vaccination available. In this context, it was recommended that efforts towards long term storage of pre-vaccination blood samples should be put in place by the company in selected areas.

A pregnancy registry should also be considered to follow up the potential risk of severe disease in early infancy. It was reflected that studying children below 9 years of age who are seropositive would be warranted.

2.5.7. Conclusions on clinical efficacy

The CYD vaccine demonstrated efficacy against symptomatic VCD in children aged 2-16 YOA living in endemic regions, with important variability across age strata, baseline serostatus strata, and according to infecting dengue serotype.

The pooled CYD14+CYD15 VE estimate against symptomatic VCD in 9-16 year olds during the whole active phase was 65.6%, and individual study results were consistent. Overall, the efficacy of the CYD dengue vaccine for each of the 4 serotypes was demonstrated but varied across serotype, with a lower VE for serotypes 1 and 2 as compared with serotypes 3 and 4. Post-hoc exploratory analyses suggest that the differences across serotypes might be confined to subjects who were dengue seronegative at baseline. The pooled CYD14 + CYD15 VE estimate in subjects from 9 to 16 years was 93.2% against clinically severe VCD and 80.8% against hospitalized VCD cases during the whole active phase (i.e. over the 25-month period after the first injection).

The 2 pivotal efficacy studies had consistent results regarding VE estimate in subjects from 9 to 16 years in relation to baseline dengue immune status, showing again that VE was higher in the dengue seropositive subjects at baseline. The vaccine has shown a clear benefit in reducing dengue disease in seropositive subjects 9 to 16 YOA (pooled CYD14+CYD15 VE of 79.4% during the PD3 period and of 81.9% during the whole active phase). The vaccine efficacy (1- Hazard Ratio), obtained from exploratory analyses (pooled CYD14 + CYD15 + CYD23) over 25-month period after the first injection, is estimated at 89.2% (95% CI: 78.5; 94.6) for hospitalized VCD and 95.3% (95% CI: 68.9; 99.3) for severe VCD. In baseline dengue seronegative subjects 9-16 YOA VE against VCD was 52.5% (95% CI: 5.9; 76.1) and inconclusive. The VE estimates in this population were also confirmed by the NS1 analysis (38% on the edge of significance).

VE was relatively stable after dose 1 and did not increase post-dose 3. VE varied also considerably according to age, with low protection levels being recorded for the youngest age group (2-5 year), but was stable above 8YOA.

In the overall population of children 2-8YOA, VE against symptomatic VCD cases over the 12-month period starting from 28 days after the third injection (PD3) due to any of the 4 serotypes was 40.5% (95% CI: 22.7; 54.2) (pooled CYD14+CYD23). Vaccine Efficacy against symptomatic, severe and hospitalized VCD during the 25-month period after the first injection due to any serotype (whole active phase, pooled CYD14+CYD23), was respectively 42.2% (95% CI: 30.6; 51.8), 45.1% (95% CI: 29.3; 76.7), 47.6% (95% CI: 23.7; 64.0).

In order to investigate the increased risk of hospitalised dengue in 2-5YOA and the effect of baseline serostatus on VE, additional post-hoc analyses were conducted to expand the dataset of subjects with a baseline serostatus assessment (NS1 analysis). Results are fully conclusive that dengue serostatus at baseline more than age is the driving factor for vaccine safety and efficacy.

Based on the NS1 supplemental study, the CYD14 VE estimate against VCD cases in seronegative subjects 2-8 years was non statistically significant with a value 18.7% (M0-M25 period, multiple imputation analysis).

The efficacy of this vaccine in seronegative individuals is measurable but data are inconclusive due to a limited number of seronegative subjects or non-statistically significant based on the NS1 analysis, and an increased risk for hospitalised dengue including clinically severe dengue (predominantly Dengue Hemorrhagic Fever grade 1 or 2) was observed regardless of age. For seronegative individuals, CYD vaccine appears to be a weak primer which induces immunity of poor quality (low neutralizing titres), rapidly waning. Also, this confirms that the risk pattern of seronegative individuals is very similar to that seen in the overall population of young children 2-5 YOA, suggesting that the excess risk in young children is actually reflecting their high seronegativity level.

Even in highly endemic countries, a substantial proportion of the population is seronegative (up to 20% on average) with important geographical and temporal differences resulting in higher proportions of seronegative individuals locally. Endemicity alone is therefore not considered an appropriate risk

minimization measure to avoid vaccination of seronegatives.

The data available support the biologically plausible argument that CYD vaccination of seronegatives mimics a primary infection and increases the risk of hospitalized and severe dengue following subsequent dengue virus exposure similar to that observed with a secondary dengue infection. The clinical profile between severe cases in seronegative subjects in the CYD group and Placebo groups were comparable. However, the immunopathogenetic mechanisms underlying the described findings are not defined. Although antibody-dependent enhancement (ADE) has been proposed as a mechanistic basis for increased risk of severe dengue upon secondary heterotypic dengue infection, there is no direct evidence from the studies conducted to conclude whether ADE or other pathogen, host or environmental factors are playing a role in the observed results.

CYD is highly immunogenic in sero-positive subjects but low neutralizing antibody titres are reached in seronegative subjects. A decrease in the GMTs against all 4 serotypes was observed one year after the third injection. Then, GMTs stabilize over the next 2 to 4 years and remain superior to pre-vaccination GMTs. Consistently with efficacy data, vaccine immunogenicity increases with age. Serotype-specific serologic response to vaccination may not consistently match with corresponding serotype-specific efficacy. GMTs levels are high after dose 2 and increase marginally with dose 3. Immunogenicity varies by country, reflecting local endemicity and serostatus at baseline.

No efficacy data were generated in individuals >16YOA and adults. The bridging of efficacy is based on all available data and overall results. Immunogenicity data available from studies in adults aged 18 to 45 years in endemic regions show that post-injection 3 GMTs against each serotype are comparable vs. GMTs in children and adolescents for whom efficacy was demonstrated in studies CYD14 and CYD15. Therefore, protection is expected in adults in endemic areas although the actual magnitude of efficacy relative to that observed in children and adolescents is unknown.

An immunological correlate of protection has not been established, but the risk of VCD decreases with increasing titres of neutralizing antibody.

Efficacy is waning over time as shown by the preliminary data observed in the SEP, with VE against symptomatic VCD in 9-16 year old is estimated 38.7% (95%CI: 11.1; 57.8) during the years 4 to 6 after the first injection. The need for and timing of a booster dose will be evaluated post-auhtorisation.

The CHMP considers that the following measures foreseen in the RMP will help to address the remaining uncertainties and missing information related to efficacy:

- The following post-authorization effectiveness studies will be conducted post-authorisation: 1) prospective study CYD69, which will estimate vaccine effectiveness against hospitalized dengue in Cebu province, the Philippines. The distribution of cases and effectiveness according to serotype will be explored as a secondary study endpoint in an effort to generate serotype-specific VE data, if variability in circulating serotypes over the 5-year duration allows. 2) Prospective case-control study DNG10042 in Parana state, Brazil to estimate effectiveness against symptomatic and hospitalized dengue where a public dengue vaccination program is taking place. 3) Studies CYD52, CYD53, and CYD70 will investigate effectiveness at the community level, as well as effectiveness at reducing frequency of hospitalization and severe forms of dengue disease. In addition, these studies will provide a platform to identify a potential increase in disease severity and a potential waning of protection over time (see RMP section 2.7). These studies will be preceded and prepared for by studies DNG13, DNG25 and DNG28 to be conducted in Malaysia, Mexico and Brazil respectively.

- Long-term follow-up data from the pivotal efficacy studies CYD14 and CYD15 will be provided as indicated in the RMP, including CYD15 final analyses on interactions between CYD vaccine exposure and Zika clinical and immunological outcomes.
- CYD63, CYD64, and CYD65 studies will investigate safety and immunogenicity of a booster dose of dengue vaccine administered in a subset of subjects who received the third dose of dengue vaccine 4-5 years before, in Phase II studies (CYD63 and CYD64). In addition, study CYD65 will also investigate immunogenicity and safety of CYD Dengue Vaccine Given in 1-, 2-, or 3-dose schedules followed by a single booster.
- Study CYD50 will generate data on CYD dengue vaccine exposure in HIV+ population.
- CYD66, CYD67, and CYD71 will investigate safety and immunogenicity of co-administration of CYD dengue vaccine with other vaccines: booster dose of Tdap (CYD66) and HPV vaccines (CYD67 and CYD71).

The CHMP recommends that the following data are submitted post-approval:

1. any available data on the effect of age and the baseline immune status on vaccine efficacy in seropositive individuals should be submitted in future PSURs.
2. any available data on (i) serotype-specific protection and (ii) the impact of serotype-specific immunity on the vaccine immunological and clinical responses should be submitted in future PSURs.

2.6. Clinical safety

Overall, regardless of age, 21 clinical studies that used CYD dengue vaccine containing the final formulation are included in the integrated safety analysis. A total of 16 studies administered CYD dengue vaccine in the final immunization schedule of 3 injections administered 6 months apart and were considered the main studies for the integrated safety analysis and 5 studies administered CYD dengue vaccine in other immunization schedule and were considered secondary studies providing supportive safety data.

A total of 4,614 subjects aged 9 to 60 years (3,067 were 9 to 17 years, and 1,547 were adults aged 18-60 years) were included in the reactogenicity subset, in which solicited injection site and systemic reactions and unsolicited AEs were assessed.

Pre-defined solicited reactions (up to 14 days) and all unsolicited reactions (up to 28 days) were assessed in the reactogenicity subset (RS). They were collected for all individuals following each injection in all studies but CYD23, CYD14 and CYD15, in which they were collected in a subset of subjects. All SAEs were collected up to at least 6 months after the last injection in studies assessing the final formulation of the CYD dengue vaccine given according to the final schedule.

While all SAEs are collected in the CYD14 and CYD15 efficacy studies up to 5 years post-injection 3, a limited set of SAEs (including related SAEs and hospitalized dengue cases) are collected in CYD05, CYD22, CYD57 and CYD28 during the long-term follow-up of safety.

The following safety endpoints were analysed:

1. in the immunogenicity and reactogenicity subset during the Active Phase:

- unsolicited systemic adverse events (AEs) reported in the 30 minutes after each dose (occurrence, nature (MedDRA preferred term), duration, intensity, action taken, and relationship to vaccination);

- solicited injection site reactions occurring up to 7 days after each dose (occurrence, time to onset, number of days of occurrence, action taken, and intensity);
- solicited systemic reactions occurring up to 14 days after each dose (occurrence, time to onset, number of days of occurrence, action taken, and intensity);
- unsolicited (spontaneously reported) AEs up to 28 days after each dose (occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to vaccination (for systemic AEs only));
- non-serious AESIs occurring up to 7 days after each dose (occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to vaccination);

2. in all subjects throughout the entire study:

- Occurrence of SAEs, including serious AESIs.

AESIs have been defined for the CYD dengue vaccine in all studies and were carefully monitored:

- Allergic reactions, including anaphylactic, as with any vaccine, within 7 days after injection;
- Acute viscerotropic or neurotropic disease (AVD, AND) within 30 days after injection: the risk of AVD and AND is linked to the surface antigens of the YF virus. As the CYD dengue vaccine has a YF backbone, AVD and AND are systematically followed as a preventive measure;
- Serious dengue diseases at any time during the study, linked to increase of severity of Dengue disease starting from the first injection related to sensitization to severe dengue disease due to vaccination.

Patient exposure

A total of approximately 28,894 subjects aged 9 months to 60 years received at least one injection of the tetravalent CYD dengue vaccine, whatever the formulation, in completed or ongoing Phase I to Phase III clinical studies including the 2 ongoing efficacy studies CYD14 and CYD15.

A total of 28,653 (of which 21,215 were 9-60YOA) received at least one injection of the final formulation, regardless of the schedule. Approximately 20,667 subjects 9 through 60 years of age received at least one injection of the final formulation of Dengvaxia according to the final vaccination schedule in 13 randomized, observer-blinded, placebo-controlled Phase II to Phase III clinical studies. Approximately 19,700 subjects aged 9 to 60 years received 3 injections of CYD dengue vaccine with the final schedule, of which 18,369 were children and adolescents. This database allows for the detection of very common, common and uncommon AEs in accordance with WHO guidelines. The database including All Studies (i.e. the 21 studies in the pooled analysis) allowed for the detection of very common, common, and uncommon AEs and SAEs that occur with an incidence ≥0.1% with at least 95% probability.

The safety profile of the CYD dengue vaccine is presented in the population 9 to 17 years of age and subjects aged 18 to 60 years. In addition, data have been analysed considering other covariates: gender, dengue immune status at baseline, and endemic versus non-endemic regions. The safety profile is based on a pooled analysis including a total of 1547 subjects 18 through 60 years of age and 19,120 subjects 9 through 17 years of age.

A total of 28,653 subjects from 9 months through 60 years received at least 1 injection of the CYD dengue vaccine (~5 log10 CCID50 per dose and per serotype, regardless of the schedule) in the 21 studies included in the integrated safety analysis. Considering the Safety Analysis Set (SafAS), a total of 27,643 subjects out of 28,653 subjects from 9 months through 60 years received at least 1 injection of the final

CYD dengue vaccine formulation at the final schedule: 1287 were infants or toddlers aged less than 2 years, 2455 were children aged 2-5 years, 12,736 were children aged 6-11 years, 9,618 were adolescents aged 12-17 years, and 1547 were adults aged 18-60 years. A total of 26,398 subjects received 3 injections of the final CYD dengue vaccine formulation at the final schedule:

- 1134 were infants or toddlers aged less than 2 years,
- 2397 children aged 2-5 years,
- 12,351 children aged 6-11 years,
- 9166 adolescents,
- 1350 adults aged 18-60 years.

A total of 80,816 injections of the CYD dengue vaccine (~5 log10 CCID50 per dose and per serotype) were administered at the final schedule. Considering the subset of subjects from SafAS who were randomized in the Reactogenicity Subset (RS) for reactogenicity assessments, a total of 7574 subjects from 9 months through 60 years received at least 1 injection of the final CYD dengue vaccine at the final schedule: 1287 were infants or toddlers aged less than 2 years, 905 were children aged 2-5 years, 2308 were children aged 6-11 years, 1527 were adolescents aged 12-17 years, and 1547 were adults aged 18-60 years.

Considering the SafAS, a total of 20,667 subjects out of 21,215 subjects from 9 years through 60 years received at least 1 injection of the final CYD dengue vaccine formulation at the final schedule: 19,120 were subjects aged 9-17 years, and 1547 adults aged 18-60 years. A total of 19,719 subjects received 3 injections of the final CYD dengue vaccine formulation at the final schedule: 18,369 subjects 9-17 years, and 1350 adults.

Considering the subjects from RS, a total of 4615 subjects from 9 years through 60 years received at least 1 injection of the final CYD dengue vaccine at the final schedule: 3067 were subjects aged 9-17 years, and 1547 were adults aged 18-60 years.

Table 27: Databases per Age Group from CYD Dengue Vaccine Recipients of the Final Formulation – Subjects Aged 9 months to 60 Years

Database	Infants and Toddlers (<2 years) N=1651	Children (2-5 years) N=2525	Children (6-11 years) N=12806	Adolescents (12-17 years) N=9689	Adults (18 to 60 years) N=1982	Total N=28653
Safety -at least one dose (regardless of schedule	1651	2525	12806	9689	1982	28653
-at least one dose (final schedule)	1287	2455	12736	9618	1547	27643
Reactogenicity (at least one dose final schedule)	1287	906	2309	1527	1547	7576
Safety follow-up post-injection 3*						
1 year	0	14134		8829	490	23453
2 years	0	7681		1700	469	9850
3 years	0	2473		171	461	3105
4 years	0	120		43	27	190
Biological parameters	179†	305		71	300	855
Viremia	269‡	301		71	311	952
Immunogenicity (final schedule)						
Non endemic region	0	0	0	0	873	873
Endemic region	1651	763	1833	1510	294	4400
Latin America	1472	100	951	949	0	2000
Asia Pacific	179	663	882	561	294	2400
Ab persistence assessment						
1 year	0	568	1393	1193	147	3301
2 years	0	101	152	169	145	567
3 years	0	98	154	166	140	558
4 years	0	98	151	159	133	541
5 Years	0	22	24	22	9	77
Efficacy §						
Phase IIb (CYD23)	0	482	2184	0	0	2666
CYD14	0	1655	3638	1555	0	6848
CYD15	0	0	6305	7609	0	13916
Phase III (CYD14 and CYD15)	0	1655	9943	9164	0	20764

Table 28: Safety data collected during long-term follow-up from CYD Dengue Vaccine Recipients of the Final Formulation

Study	Number of subjects who received the CYD dengue vaccine	Planned total duration of follow-up	Data collected during the long-term follow-up		Data included in the integrated analysis
			SAEs	Dengue cases	
CYD05	123	5 years after the last injection	From 6 months after the last injection to 4 years after the last injection: all SAEs (except accidents, hospitalizations due to preexisting conditions and planned surgeries) and deaths From 4 years to 5 years after the last injection: SAEs upon Investigator's judgment	From 1 year after the last injection to 4 years after the last injection: dengue cases From 4 years to 5 years after the last injection: hospitalized dengue cases	All data: 5 years after the last injection
CYD22	120	4 years after the last injection	From 6 months after the last injection to 4 years after the last injection: related SAEs and fatal SAEs	From 1 year after the last injection to 4 years after the last injection: dengue cases	Data until 4 years after the last injection
CYD28	898	4 years after the last injection	From 6 months after the last injection to 4 years after the last injection: related SAEs and fatal SAEs	From 1 year after the last injection to 4 years after the last injection: hospitalized dengue cases	Data until 4 years after the last injection
CYD23 + CYD57*	2668	5 years after the last injection	From 6 months after the last injection to 5 years after the last injection: related SAEs and fatal SAEs	From 1 year to 5 years after the last injection: hospitalized dengue cases	Data until 3 years after the last injection
CYD14	6849	5 years after the last injection	From 6 months after the last injection to 5 years after the last injection: all SAEs	From 1 year after the last injection to 5 years after the last injection: hospitalized dengue cases	Data until 2 years after the last injection
CYD15	13918	5 years after the last injection	From 6 months after the last injection to 5 years after the last injection: all SAEs	From 1 year after the last injection to 5 years after the last injection: hospitalized dengue cases	Data until 2 years after the last injection

* CYD57 data are not included in the pooled analysis

Table 29: Number of Subjects Followed per Completed Year of Long-Term Safety Follow-Up

Safety follow-up post-injection 3	Studies included	Estimated Number of subjects (n)*			
		Children (2-11 y)	Adolescents (12-17 y)	Adults (18-45 y)	Total
1-year Follow-up	CYD05, CYD22, CYD28, CYD23/57, CYD14, CYD15	14,134	8,829	490	23,453
2-year Follow-up†	CYD05, CYD14, CYD22, CYD28, CYD23/57, CYD15	13,612	8,693	469	22,774
3-year Follow-up‡	CYD05, CYD22, CYD28, CYD23/57	2,473	171	461	3,105
4-year Follow-up§	CYD05, CYD22, CYD28	338	168	451	957

* Number of subjects calculated based on the following rules: real yearly number of subjects who received 3 injections of CYD dengue vaccine ~5 $\log_{10}$ $CCID_{50}$ of serotypes 1, 2, 3 and 4 when study completed.
† Corresponds to Year 1 Hospital Surveillance/Phase in CYD23/57, CYD14 and CYD15.
‡ Corresponds to Year 2 Hospital Surveillance in CYD23/57.
§ Year 3 Hospital Surveillance in CYD23/57 is completed but as the database is not locked and not analyzed (as per protocol) at the time of the present Application, no number of subjects followed during this year is presented in this table.

Adverse events

Solicited local symptoms

Solicited injection site reactions after any CYD dengue vaccine injection are presented in Table 30 by age group in 9 through 60 year-olds in the reactogenicity subset in main safety studies.

Table 30: Solicited injection site reactions after any CYD dengue vaccine injection during the solicited period – Subjects Aged 9 Years and Above – RS Main Studies Pooled

	Adults (18 to 60 years)			Subjects 9 to 17 years		
Subject with at least one	n/M	%	(95% CI)	n/M	%	(95% CI)
Solicited injection site reaction	714/1524	46.9	(44.3; 49.4)	1556/3050	51.0	(49.2; 52.8)
Grade 3 solicited injection site reaction	11/1524	0.7	(0.4; 1.3)	45/3050	1.5	(1.1; 2.0)
Pain	689/1524	45.2	(42.7; 47.7)	1502/3050	49.2	(47.5; 51.0)
Erythema	120/1524	7.9	(6.6; 9.3)	255/3049	8.4	(7.4; 9.4)
Swelling	37/1524	2.4	(1.7; 3.3)	209/3050	6.9	(6.0; 7.8)

n: number of subjects experiencing the endpoint; M: number of subjects with available data for the relevant endpoint

Solicited injection site reactions were reported in approximately half of the subjects with a low proportion experiencing Grade 3 reactions in subjects aged 9 years and over. Of all solicited injection site reactions, less than 1% were Grade 3.

In adult subjects, solicited injection site reactions were more frequently reported in the Dengue Group than in the placebo group, whereas similar trends were observed in the Placebo and Dengue Groups in subjects aged 9 to 17 years. The occurrence of solicited injection site reactions was similar in adults and subjects aged 9 to 17 years in the Dengue Group.

Injection site pain was the most commonly reported reaction (more than 45% of subjects). The majority of these reactions was of Grade 1 intensity and resolved within 3 days without sequelae. Grade 3 reactions occurred at a rate below 1.5% in all age groups and were of short duration (<3 days) and reversible (Table 30). Rates of solicited injection site reactions remained similar after each successive injection.

Similar trends were observed in children 2 to 11 years, in which reactogenicity tended to be more frequent than in adults in both the CYD dengue and placebo groups. In toddlers (Dengue Groups only), the occurrence of solicited injection site reactions tended to be lower compared to other age groups.

Table 31: Solicited injection site reactions after any CYD dengue vaccine or placebo dose by maximum intensity during the solicited period – Subjects 9-17 years – RS Main Studies Pooled

		CYD dengue vaccine			Placebo		
Subjects experiencing at least one:	Maximum intensity	n/M	%	(95% CI)	n/M	%	(95% CI)
Pain		**1502/3050**	**49.2**	**(47.5; 51.0)**	**574/1470**	**39.0**	**(36.5; 41.6)**
	Grade 1	1232/3050	40.4	(38.6; 42.2)	486/1470	33.1	(30.7; 35.5)
	Grade 2	229/3050	7.5	(6.6; 8.5)	76/1470	5.2	(4.1; 6.4)
	Grade 3	41/3050	1.3	(1.0; 1.8)	12/1470	0.8	(0.4; 1.4)
Erythema		**255/3049**	**8.4**	**(7.4; 9.4)**	**110/1470**	**7.5**	**(6.2; 8.9)**
	Grade 1	230/3049	7.5	(6.6; 8.5)	106/1470	7.2	(5.9; 8.7)
	Grade 2	24/3049	0.8	(0.5; 1.2)	3/1470	0.2	(0.0; 0.6)
	Grade 3	1/3049	<0.1	(0.0; 0.2)	1/1470	<0.1	(0.0; 0.4)
Swelling		**209/3050**	**6.9**	**(6.0; 7.8)**	**75/1470**	**5.1**	**(4.0; 6.4)**
	Grade 1	186/3050	6.1	(5.3; 7.0)	69/1470	4.7	(3.7; 5.9)
	Grade 2	20/3050	0.7	(0.4; 1.0)	5/1470	0.3	(0.1; 0.8)
	Grade 3	3/3050	<0.1	(0.0; 0.3)	1/1470	<0.1	(0.0; 0.4)

Table summarizes worst case for a subject which is the maximum intensity observed from each dose
n: number of subjects experiencing the endpoint
M: number of subjects with available data for the relevant endpoint
CYD dengue vaccine ~5 $\log_{10}$ $CCID_{50}$ of serotypes 1, 2, 3 and 4
Main studies applied a D0/M6/M12 vaccine schedule
Contributing studies: CYD13 CYD14 CYD15 CYD22/CYD23 CYD24 CYD28 CYD30 CYD32

Solicited systemic reactions

Solicited systemic reactions after any CYD dengue vaccine injection are presented in Table 32 by age group. They consist of clinical objective and subjective signs.

Table 32: Solicited systemic reactions after any CYD dengue vaccine injection

	Adults (18 to 60 years)			Subjects aged 9 to 17 years		
Subject with at least one	**n/M**	**%**	**(95% CI)**	**n/M**	**%**	**(95% CI)**
Solicited systemic reaction	999/1524	65.6	(63.1; 67.9)	2043/3050	67.0	(65.3; 68.7)
Grade 3 solicited systemic reaction	164/1524	10.8	(9.2; 12.4)	338/3050	11.1	(10.0; 12.2)
Fever	75/1522	4.9	(3.9; 6.1)	500/3040	16.4	(15.1; 17.8)
Headache	784/1524	51.4	(48.9; 54.0)	1649/3048	54.1	(52.3; 55.9)
Malaise	675/1524	44.3	(41.8; 46.8)	1247/3047	40.9	(39.2; 42.7)
Myalgia	643/1524	42.2	(39.7; 44.7)	1280/3047	42.0	(40.2; 43.8)
Asthenia	432/1524	28.3	(26.1; 30.7)	1042/3047	34.2	(32.5; 35.9)

Overall, solicited systemic reactions tended to decrease after each successive injection. The most common solicited systemic reactions were headache (>50%), malaise (>40%), myalgia (>40%) and asthenia (<35%). Over 60% of participants reported a solicited systemic reaction, of which approximately 10% were Grade 3. Most Grade 3 solicited reactions were related to headache or fever.

Fever occurred less frequently than headaches (approximately 16% in subjects aged 9 to 17 years and less than 5% in adults) but tended to occur throughout the observation period for solicited reactions.

In the Placebo Group, the incidence of each solicited systemic reaction was comparable to that of the Dengue Group for subjects aged 9 to 17 years, whereas incidence was slightly higher in the Dengue Group than in the Placebo group in adults. However, regardless of age, the time to onset and number of days of occurrence were similar in the Dengue Group and Placebo Group.

Similar trends were observed in adolescents (12 to 17 years), in children (2 to 11 years -Table 34) and in toddlers (<2 years of age) as in subjects aged 9 to 17 years (Table 33).

Table 33. Solicited systemic reactions after any CYD dengue vaccine or placebo dose by maximum intensity during the solicited period – Subjects 9-17 years – RS Main Studies Pooled

		CYD dengue vaccine			Placebo		
Subjects experiencing at least one:	**Maximum intensity**	**n/M**	**%**	**(95% CI)**	**n/M**	**%**	**(95% CI)**
Fever		**500/3040**	**16.4**	**(15.1; 17.8)**	**228/1465**	**15.6**	**(13.7; 17.5)**
	Grade 1	242/3040	8.0	(7.0; 9.0)	119/1465	8.1	(6.8; 9.6)
	Grade 2	167/3040	5.5	(4.7; 6.4)	76/1465	5.2	(4.1; 6.5)
	Grade 3	91/3040	3.0	(2.4; 3.7)	33/1465	2.3	(1.6; 3.1)
Headache		**1649/3048**	**54.1**	**(52.3; 55.9)**	**762/1471**	**51.8**	**(49.2; 54.4)**
	Grade 1	979/3048	32.1	(30.5; 33.8)	454/1471	30.9	(28.5; 33.3)
	Grade 2	475/3048	15.6	(14.3; 16.9)	239/1471	16.2	(14.4; 18.2)
	Grade 3	195/3048	6.4	(5.6; 7.3)	69/1471	4.7	(3.7; 5.9)
Malaise		**1247/3047**	**40.9**	**(39.2; 42.7)**	**552/1471**	**37.5**	**(35.0; 40.1)**
	Grade 1	783/3047	25.7	(24.2; 27.3)	357/1471	24.3	(22.1; 26.5)
	Grade 2	340/3047	11.2	(10.1; 12.3)	154/1471	10.5	(9.0; 12.1)
	Grade 3	124/3047	4.1	(3.4; 4.8)	41/1471	2.8	(2.0; 3.8)
Myalgia		**1280/3047**	**42.0**	**(40.2; 43.8)**	**560/1471**	**38.1**	**(35.6; 40.6)**
	Grade 1	826/3047	27.1	(25.5; 28.7)	375/1471	25.5	(23.3; 27.8)
	Grade 2	351/3047	11.5	(10.4; 12.7)	154/1471	10.5	(9.0; 12.1)
	Grade 3	103/3047	3.4	(2.8; 4.1)	31/1471	2.1	(1.4; 3.0)
Asthenia		**1042/3047**	**34.2**	**(32.5; 35.9)**	**460/1471**	**31.3**	**(28.9; 33.7)**
	Grade 1	653/3047	21.4	(20.0; 22.9)	301/1471	20.5	(18.4; 22.6)
	Grade 2	286/3047	9.4	(8.4; 10.5)	120/1471	8.2	(6.8; 9.7)
	Grade 3	103/3047	3.4	(2.8; 4.1)	39/1471	2.7	(1.9; 3.6)

Table summarizes worst case for a subject which is the maximum intensity observed from each dose
n: number of subjects experiencing the endpoint
M: number of subjects with available data for the relevant endpoint
CYD dengue vaccine ~5 log10 CCID50 per dose of serotypes 1, 2, 3 and 4
Main studies applied a D0/M6/M12 vaccine schedule
Contributing studies: CYD13 CYD14 CYD15 CYD22 CYD23 CYD24 CYD28 CYD30 CYD32

Table 34. Solicited systemic reactions after any CYD dengue vaccine or placebo dose by maximum intensity during the solicited period - Children (2-11 years) - RS Main Studies Pooled

		CYD dengue vaccine			Placebo		
Subjects experiencing at least one:	Maximum intensity	n/M	%	(95% CI)	n/M	%	(95% CI)
Fever		635/3192	19.9	(18.5; 21.3)	259/1503	17.2	(15.4; 19.2)
	Grade 1	315/3192	9.9	(8.9; 11.0)	129/1503	8.6	(7.2; 10.1)
	Grade 2	204/3192	6.4	(5.6; 7.3)	85/1503	5.7	(4.5; 6.9)
	Grade 3	116/3192	3.6	(3.0; 4.3)	45/1503	3.0	(2.2; 4.0)
Headache		1577/3200	49.3	(47.5; 51.0)	675/1504	44.9	(42.3; 47.4)
	Grade 1	1099/3200	34.3	(32.7; 36.0)	458/1504	30.5	(28.1; 32.8)
	Grade 2	357/3200	11.2	(10.1; 12.3)	167/1504	11.1	(9.6; 12.8)
	Grade 3	121/3200	3.8	(3.1; 4.5)	50/1504	3.3	(2.5; 4.4)
Malaise		1337/3199	41.8	(40.1; 43.5)	554/1504	36.8	(34.4; 39.3)
	Grade 1	936/3199	29.3	(27.7; 30.9)	389/1504	25.9	(23.7; 28.2)
	Grade 2	315/3199	9.8	(8.8; 10.9)	133/1504	8.8	(7.5; 10.4)
	Grade 3	86/3199	2.7	(2.2; 3.3)	32/1504	2.1	(1.5; 3.0)
Myalgia		1233/3199	38.5	(36.9; 40.3)	519/1504	34.5	(32.1; 37.0)
	Grade 1	912/3199	28.5	(26.9; 30.1)	398/1504	26.5	(24.2; 28.8)
	Grade 2	248/3199	7.8	(6.8; 8.7)	96/1504	6.4	(5.2; 7.7)
	Grade 3	73/3199	2.3	(1.8; 2.9)	25/1504	1.7	(1.1; 2.4)
Asthenia		1028/3199	32.1	(30.5; 33.8)	438/1504	29.1	(26.8; 31.5)
	Grade 1	699/3199	21.9	(20.4; 23.3)	316/1504	21.0	(19.0; 23.2)
	Grade 2	260/3199	8.1	(7.2; 9.1)	90/1504	6.0	(4.8; 7.3)
	Grade 3	69/3199	2.2	(1.7; 2.7)	32/1504	2.1	(1.5; 3.0)

Unsolicited AEs

Slightly less than one half of subjects receiving dengue vaccine reported an unsolicited AE (from 44.2 to 46.2% of subjects). These were primarily medical conditions commonly seen for the age groups described (9-17 and 18-45 years of age) and were mostly not severe and unrelated to vaccination. The incidence of unsolicited non-serious AEs tended to decrease with subsequent injections. Most unsolicited non-serious AEs were of Grade 1 and 2 intensity. Grade 3 AEs were reported by 5.4% of subjects aged 9 to 17 years and by 8.5% of adult subjects.

In adults, 11.6% of subjects had at least one unsolicited AE related to injection by the Investigators, whereas in subjects aged 9 to 17 years, 2.2% of subjects had at least one unsolicited AE assessed as related to injection. The nature of these AEs in terms of SOCs and PTs can be expected given the age group of the subjects. The most frequently reported non-serious unsolicited AEs were in the SOCs Infections and infestations, Respiratory, thoracic and mediastinal disorders, Gastrointestinal disorders, General disorders and administration site conditions, Nervous system disorders, in subjects aged 9 to 17 years and in adults, and Musculoskeletal and connective tissue disorders, Injury, poisoning and procedural complications in adults only. The incidence was <3% in the remaining SOCs.

Analysis of SOCs corresponding to reported reactions showed no clinically relevant differences between the CYD dengue vaccine and placebo. Each individual reaction was reported at a frequency below 3%.

In adults and in subjects aged 9 to 17 years, non-serious unsolicited adverse reactions (ARs) were mostly Grade 1 or 2. Less than 1.5% of subjects (1.3% in adults and 0.2% in subjects aged 9 to 17 years) had an unsolicited AR of Grade 3 severity.

There were no safety concerns related to the nature and frequency of unsolicited AEs.

Similar trends were observed in adolescents (12 to 17 years), children (2 to 5 years and 6 to 11 years) and infants and toddlers (Dengue Groups only) as in subjects aged 9 to 17 years.

Conclusion on related AEs

For potential allergic reactions within 7 days, reactions of all seriousness and rash of all natures have been considered to calculate the frequency of these events:

A total of 6 cases of rash have been reported in adults, including 2 cases of rash generalized, 3 cases of rash and 1 case of rash erythematous (6/1547, uncommon); 2 cases have been reported in children aged from 9 years (rash and rash maculo-papular) (2/3068, rare);

A total of 4 cases of urticaria have been reported in children aged from 9 years, including one related SAE (with a history of allergic rhinitis) (4/3068, Uncommon).

For the other related SAEs, they were isolated in terms of nature and frequency (only 1 subject for each, which corresponds to a frequency below the level of detection of the safety database i.e., > 0.1%).

The reactions listed in the SmPC are the following in subjects aged 9 to 45 years:

Very common (≥10%): headache, myalgia, injection site pain, malaise, asthenia and fever

Common (≥1% and <10%): Injection site reactions (erythema, swelling)

Uncommon (≥0.1% and <1%): lymphadenopathy, upper respiratory tract infection, dizziness, Migraine, oropharyngeal pain, cough, rhinorrhoea, nausea, dry mouth, (generalised) rash, urticaria, neck pain, arthralgia, injection site induration and warmth, injection site reactions (hematoma, pruritus), chills, fatigue.

The most frequently reported adverse reactions (Very common and Common) are similar for children aged 9 to 17 years and for adults, with few differences in terms of frequency, i.e. fever was less frequently reported in adults (common) and injection site hematoma and pruritus was less frequently reported in children aged 9 to 17 years (Uncommon).

Regarding the uncommon adverse reactions, age group specificities have been observed: lymphadenopathy, migraine, arthralgia and were reported only in adults, urticaria was only reported in subjects aged 9 to 17 years, and upper respiratory tract infection, dizziness, oropharyngeal pain, cough, rhinorrhoea, nausea, rash and neck pain were less frequently reported in subjects aged 9 to 17 years (rare or very rare).

The safety profile of the CYD dengue vaccine was acceptable within 6 months post any injection in all the populations studied, i.e. in all age groups and regions (non-endemic, endemic Asia Pacific, or endemic Latin America), and irrespective of gender and dengue, FV, JE or YF status at baseline based on post-hoc analyses.

Approximately 28,600 subjects aged 9 months to 60 years received at least 1 injection of the final formulation, regardless of the schedule. Among these subjects, 21,215 subjects were in the target age indication (9 to 60 years of age). The majority of the subjects are children and adolescents with 1982 adults aged 18 to 60 years of which 241 were over 45 years receiving at least one dose. The database allows for the detection of very common, common and uncommon AEs, i.e. incidence ≥0.1%, in accordance with WHO guidelines (48). The majority of subjects have been followed for safety for at least 1 year while all of the subjects enrolled in the 3 efficacy studies will be evaluated for safety and the occurrence of SAEs (all SAEs in CYD14 and CYD15 and related SAEs in CYD57) and hospitalized VCD for 5 years post-injection 3 with the provision of regular safety reports in an ongoing basis (please refer to section 4.4).

The methodology and outcome measures for the safety database were appropriate and similar across all clinical studies so that data could be pooled for analysis which increased the power for the detection of safety signal. The data demonstrated that the reactogenicity profile after any injection of the CYD dengue vaccine is similar to licensed vaccines used in the age groups that have been studied and also similar when compared to placebo. Unsolicited AEs reported during the 28-day monitoring period after each injection were common medical conditions normally observed in these age groups and occurred with similar frequency compared to control groups.

The safety profile of the CYD dengue vaccine in terms of incidence, severity, and nature of events was generally similar to that reported after injection of placebo, although in adults, the incidence of several clinical safety parameters had higher incidence in the Dengue Group than in the Placebo Group. The safety profile of the CYD dengue vaccine (reactogenicity) was found to be similar to that of comparator vaccines, i.e., different licensed vaccines (MMR, YF, dTaP-IPV/Hib, PCV13) mainly used as benefit vaccines or as part of the vaccination schedules of the under 5 years age groups. No data are available concerning the claimed target population of 9 years and above. This is addressed in section 4.5 of the SmPC.

Serious adverse event/deaths/other significant events

Deaths

Deaths were reported with a similar frequency in both Dengue and Control Groups. No deaths were assessed as related to the study vaccine in any study.

A total of 10 fatal cases due to dengue disease occurred after vaccination as reported from the worldwide post-marketing setting (including public programs in the Philippines and the Parana state of Brazil) (see section 2.6.1).

Serious adverse events (non-Dengue related)

SAEs within 28 days after any injection were reported in approximately 1% of subjects (between 0.6% and 1.8% depending on the age group), and were mainly diseases, infections or injuries commonly reported in these age groups, and no cluster in terms of nature and frequency was observed.

In the Phase 3 trials, the number of serious adverse events (SAEs) was similar between CYD and placebo group. Related SAEs up to 28 days after a CYD injection occurred in 6 subjects (headache and polymyalgia rheumatic in adults, and allergic urticarial-asthma, acute polyneuropathy, ADEM and tension headache in 9-17 year-old participants). An additional SAE was classified as related by the investigator in the 28 days to 6 months post CYD injection (blighted ovum), and 1 SAE of convulsion was judged to be related by the sponsor (not the Investigator). For ADEM, acute polyneuropathy and convulsion, no vaccine viruses were isolated from the subjects.

No safety concerns were identified during long-term follow-up of all studies having a long-term follow-up (Cut-off date on 01 September 2015), as no evidence of excess of any specific SAEs were reported. In particular, no related SAEs were reported in the Dengue Group.

Overall, there is no evidence of an association between CYD-TDV and non-dengue serious adverse events based on clinical trials for the population aged 9-60 years. There are a limited number of trial participants beyond 16 years of age to assess the risk of serious adverse events in the 18-45 year population. Even for the 9-16 year-olds, the population included in the Phase 3 trials, risks of rare serious adverse events would require further assessment in post-licensure studies, as for any other vaccine.

Adverse Events of Special Interests (AESIs)

The following AESIs have been defined by the Applicant: allergic reactions within 7 days after vaccination, acute viscerotropic or neurotropic disease (AVD, AND) with 30 days after vaccination, and serious dengue disease at any time during the study.

Allergic reactions

They were selected as AESIs for the CYD dengue vaccine since, as with any vaccine, a risk of allergic reaction with the CYD dengue vaccine, in terms of rash, urticaria, severe asthma, or shock, is possible but very rare. Allergic reactions were collected in all studies as unsolicited AEs including SAEs. For the purposes of the pooled/integrated analysis, and in order to ensure homogeneity, anaphylactic reactions were identified and analysed in all studies based on the Standardized MedDRA Query (SMQ) algorithm to detect potential anaphylactic reactions. In addition, a pre-defined targeted list of PTs was used in order to detect potential systemic allergic reactions. As a result, the pooled/integrated analysis may provide different results than those reported in the individual CSRs.

No immediate anaphylactic shock has been reported post-vaccination. Five subjects receiving CYD have experienced a serious potential allergic reaction: 4 subjects with asthma/asthmatic crisis (all had medical history), and 1 urticaria (with history of allergic rhinitis). In the placebo group, there was one serious adverse event suggestive for allergic reaction (asthma in a subject with a history of asthma).

Viscerotropic and neurotropic events

These events were selected as AESIs because a YF-17D replicating engine is used as construct of the CYD dengue vaccine, and based on the assumption that vaccination with a YF-17D vaccine could be associated with the extremely rare occurrence of acute viscerotropic and neurotropic diseases (within 30 days after vaccine injection). Guidelines for the early detection and evaluation of suspected cases of viscerotropic and neurotropic diseases, including assessment of vaccine virus replication and of differential diagnosis, were provided to the Investigators in all clinical studies. These guidelines were written in accordance with the Centre for Disease Control's (CDC) case definitions for YF vaccine-associated viscerotropic disease (YEL-AVD) and neurtropic disease (YEL-AND). The guidelines were updated during the clinical development program to reflect the Brighton collaboration recommendation for a risk window for YEL-AVD of 30 days instead of 10 days. Therefore, the time to onset for suspected viscerotropic disease was extended from 10 days to 30 days. Events reaching level 2 of viscerotropism or neurotropism were to be reported as SAEs.

10 SAEs occurring within 30 days from any injection were reported as suspected neurotropism or viscerotropism cases and were further investigated, in particular in the Dengue Group. In all biological specimens from these subjects, genomic amplification was negative for vaccine virus and/or WT YF virus strains. None of these suspected cases were confirmed as neurotropic or viscerotropic disease.

Biological specimens from 9 subjects were tested for neurotropism:

- 3 in the Dengue Group for whom the PTs of the final diagnosis were ADEM (CYD14), acute polyneuropathy (CYD15), and convulsion (CYD15).
- 1 in Placebo Group, who received yellow fever vaccine concomitantly with the placebo, for whom the PT of the final diagnosis was viral meningitis, followed by acute cerebellitis (CYD29).
- 5 in the Placebo Group for whom the PTs of the final diagnosis were convulsion (CYD14), encephalitis viral (CYD14), visual impairment (CYD15), VIIth nerve paralysis (CYD32), and meningitis viral (CYD28).

Biological specimens of 1 subject were tested for viscerotropism in the Dengue Group, for whom the PT of the initial diagnosis was Guillain-Barre Syndrome and the final diagnosis was leptospirosis (CYD15).

Overall, no events of viscerotropic or neurotropic disease were observed after administration of the CYD dengue vaccine in any studies. There have been no confirmed AVD or AND cases in the studies. No risk of viscerotropism, neurotropism or sensitization to severe disease was identified from non-clinical pharmacology either. However, considering the characteristics of both the attenuated vaccine and the parent viruses (i.e. wild-type dengue and YF 17D viruses), theoretical risks may exist. These risks were closely monitored during clinical trials (including long term follow-up), and will continue to be monitored once the vaccine is licensed.

Severe dengue disease

In agreement with WHO guidelines, the Company has gathered data to determine that the immune response to the vaccine does not predispose vaccinated individuals to develop severe dengue following natural infections in endemic regions. Thus, SVCD cases were followed closely in clinical studies with CYD dengue vaccine.

The Company has determined the density incidence of SVCD in the Dengue vaccinated group and in the control group, and then calculated the Relative risk of SVCD in vaccinated subjects to those who received placebo. The Applicant has made these calculations for each of the three individual efficacy studies (CYD14, CYD15 and CYD23), as well as for the pool of the three trials. These data have been stratified according the Dengue serotype causing the SVCD case, and in different age groups (all subjects 2 to 16 YOA; claimed indication 9-16 YOA, 12/14 to 16 YOA, 2-5 YOA and 6 to 11 YOA).

For the analyses of SVCD performed in the Active Phase, and considering that RR can be basically converted to Vaccine efficacy against SVCD by applying the formula VE= (1-RR)x100, it should be mentioned that the assessment made in the efficacy section of this report regarding SVCD VE also apply to this safety section regarding RR of SVCD.

With respect to the data described in this safety section, in the three trials there was no excess of SVCD cases during the Active Phase (25-month following the first injection) in the Dengue Group compared to the Control Group regardless of the age category.

Active Phase

During the Active Phase, no increase of risk of SVCD disease was observed. There was no excess of SVCD due to any serotype in subjects in the Dengue Group compared to the Control Group regardless of the age of the population in the 3 efficacy trials CYD14, CYD15, and CYD23. In both adolescents and children, SVCD occurred with a low and similar density incidence in the endemic AP and endemic LatAm regions, and there was no excess of SVCD in the Dengue Group compared to the Control Group in the 2 endemic regions AP and LatAm.

The impact of age group, gender, region was limited to trends toward differences in incidences of safety parameters during the active phase for the >9 years of age. As the trends were generally also observed in the Placebo Group, an impact of reporting practices in the various populations was suspected.

Hospital Phase

Long-term follow-up (LTFU) was defined as the period from Month 6 after the last injection onward for SAEs and from Year 1 after the last injection onward for dengue cases (designated as Surveillance/Hospital Phase).

The pooled data from the three trials CYD14+CYD15+CYD57 up to Year 4 Hospital Phase in CYD57 and Year 2 Hospital Phase in CYD14 and CYD15 indicated that the incidence of hospitalized VCD cases during Year 1 and Year 2 Hospital Phase was, for the age group 9-16 years, significantly lower in the Dengue group compared to the Control group (RR=0.5 [95%CI: 0,28; 0.89] and RR=0.562 [95%CI: 0.32; 1.00]) respectively, indicating VE. For the age group 2-8 years, the hospitalized VCD cases tended to be higher in the Dengue group compared to the control group at Year 1 HP (RR=1.576 [95%CI: 0.81; 3.31]. Moreover, for the serotype 2 in this age group, the RR was statistically higher than 1 (RR= 7.964 [95%CI: 1.24; 333.98], 16 cases in the Dengue group vs 1 case in the control group), which indicates that more hospitalized VCD due to serotype 2 occurred in the vaccinated than in the control group. When these data were analysed per individual trial according to different age groups, the unbalance was confined to the age group 2-5 years (Y1 of CYD14), since there were 15 cases in the Dengue group and 1 in the control, which resulted in a RR = 7.454 (1.15-313.80) (with the 95%CI not including 1).

When looking at the data available as of September 2016 gathered during the LTFU, there appears to be a similar unexpected result in several of the age group. In trial CYD57, for the age group 9-11 y, there are 11 and 5 hospitalized VCD cases in the dengue and control group, respectively at Y4 HP (RR= 1.120 (0.36; 4.11)).

Table 35: Incidence of hospitalised VCD cases during the entire study by age group - Efficacy Studies Integrated Pooled SafAS

				CYD Dengue Vaccine Group (N=23433)				Control Group (N=11690)				Relative Risk	
Study	Serotype	Age Group	Time period	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	RR	(95% CI)
CYD23/57	Any serotypes	9-11 year olds	Year 1 (D0 to Injection 3)	3	1033	0.3 (0.1; 0.8)	3	2	521	0.4 (0.0; 1.4)	2	0.757	(0.09; 9.06)
			Year 2 (Injection 3 to the end of Active Phase)	3	1005	0.3 (0.1; 0.8)	3	9	508	1.6 (0.8; 3.1)	9	0.168	(0.03; 0.68)
			Active Phase	6	1019	0.3 (0.1; 0.6)	6	11	515	1.0 (0.5; 1.8)	11	0.275	(0.08; 0.81)
			Year 3 (first year of Hospital Phase)	3	794	0.4 (0.1; 1.2)	3	5	406	1.3 (0.4; 3.1)	5	0.307	(0.05; 1.58)
			Year 4 (second year of Hospital Phase)	3	794	0.4 (0.1; 1.1)	3	5	406	1.2 (0.4; 2.9)	5	0.307	(0.05; 1.58)
			Year 5 (third year of Hospital Phase)	1	781	0.1 (0.0; 0.7)	1	3	401	0.7 (0.2; 2.2)	3	0.171	(0.00; 2.13)
			Year 6 (fourth year of Hospital Phase)	11	768	1.4 (0.7; 2.5)	11	5	391	1.3 (0.4; 3.0)	5	1.120	(0.36; 4.11)
			Hospital Phase	18	784	2.3 (1.4; 3.6)	18	18	401	4.5 (2.7; 7.0)	18	0.511	(0.25; 1.04)
			Entire Study	24	863	0.5 (0.3; 0.7)	24	29	439	1.1 (0.7; 1.6)	29	0.421	(0.23; 0.75)

In trial CYD14, for the age group 9-11 y, there were 12 and 1 hospitalized VCD cases in the dengue and control group, at Y2 HP (RR=6.028 (0.89; 257.67)). Overall, the RR was <1 (RR=0.219 (0.08; 0.38)) during the active phase and was above 1 for the hospital phase (RR=2.266 (0.75; 9.20)).

Table 36: Incidence of hospitalised virologically-confirmed dengue cases during the entire study by age group – Efficacy Studies Integrated/Pooled SafAS

				CYD Dengue Vaccine Group (N=23433)				Control Group (N=11690)				Relative Risk	
Study	Serotype	Age Group	Time period	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	RR	(95% CI)
CYD14	Any serotypes	9-11 year olds	Year 1 (D0 to Injection 3)	5	1761	0.3 (0.1; 0.7)	5	4	882	0.5 (0.1; 1.2)	4	0.626	(0.13; 3.16)
			Year 2 (Injection 3 to the end of Active Phase)	2	1751	0.1 (0.0; 0.4)	2	12	879	1.3 (0.7; 2.2)	12	0.084	(0.01; 0.38)
			Active Phase	7	1756	0.2 (0.1; 0.4)	7	16	881	0.9 (0.5; 1.4)	16	0.219	(0.08; 0.56)
			Year 3 (first year of Hospital Phase)	6	1742	0.4 (0.1; 0.8)	6	3	877	0.4 (0.1; 1.1)	3	1.007	(0.22; 6.22)
			Year 4 (second year of Hospital Phase/SEP)	12	1738	0.7 (0.4; 1.2)	12	1	873	0.1 (0.0; 0.6)	1	6.028	(0.89; 257.67)
			Hospital Phase	18	3326	0.5 (0.3; 0.9)	18	4	1675	0.2 (0.1; 0.6)	4	2.266	(0.75; 9.20)
			Surveillance Expansion Period	-	-		-	-	-		-	NC	(NC)
			Hospital Phase/SEP	18	1740	0.5 (0.3; 0.9)	18	4	875	0.2 (0.1; 0.6)	4	2.263	(0.75; 9.19)
			Entire Study	25	1748	0.4 (0.2; 0.5)	25	20	878	0.6 (0.3; 0.9)	20	0.628	(0.33; 1.19)

To assess the overall long-term safety and efficacy of the vaccine, the Applicant provided an analyses of the RR of hospitalized VCD and SVCD cases detected from the first injection till the end of each of the four years of hospital phase, showing that over the observed period, and for all trials, the RR remains <1.

Nonetheless, there is a slow increase of RR overtime in all studies potentially suggesting a slight waning of efficacy but RR remains <1. Based on the available data, the above analyses confirmed the decreased risk of hospitalized VCD cases in vaccinees versus placebo recipients over time, i.e. since first injection up to the end of Year 2 (CYD14 and CYD15) or Year 6 (CYD23/57). The same trend for decreased risk is also observed for SVCD in vaccinees versus placebo recipients up to the end of Year 2 in CYD15 and CYD14. Due to the limited number of cases reported in these studies, the conclusions on the risk of SVCD overtime are to be considered with caution, especially in CYD23/57.

Table 37: Incidence and relative risk of hospitalised virologically confirmed dengue cases due to any serotype from Injection 3 to end of the Active Phase and from Injection 1 to end of each year of follow up – Efficacy Studies Integrated SafAS

		Dengue Group (N=23433)				Control Group (N=11690)				Relative Risk	
Study	Time period	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	RR	(95% CI)
CYD14	From Injection 1 to 28 days PD3 (1 year post-dose 1)	20	6849	0.3 (0.2; 0.5)	20	26	3423	0.8 (0.5; 1.1)	26	0.384	(0.20; 0.72)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	20	6 813	0.3 (0.2; 0.4)	20	35	3406	0.9 (0.7; 1.3)	35	0.286	(0.16; 0.51)
	From Injection 1 to end of Active phase (25 months post-dose 1)	40	6831	0.3 (0.2; 0.4)	40	61	3415	0.9 (0.7; 1.1)	61	0.328	(0.21; 0.50)
	From Injection 1 to end of Year 3 (first year of Hospital Phase)	67	6814	1.0 (0.8; 1.2)	67	73	3405	2.1 (1.7; 2.7)	74	0.459	(0.32; 0.65)
	From Injection 1 to end of Year 4 (second year of Hospital Phase / SEP)	124	6799	1.8 (1.5; 2.2)	124	99	3398	2.9 (2.4; 3.5)	101	0.626	(0.48; 0.82)
CYD23/57	From Injection 1 to 28 days PD3 (1 year post-dose 1)	8	2668	0.3 (0.1; 0.6)	8	7	1329	0.5 (0.2; 1.1)	7	0.569	(0.18; 1.84)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	24	2578	0.9 (0.6; 1.3)	24	23	1290	1.6 (1.0; 2.5)	23	0.522	(0.28; 0.97)
	From Injection 1 to end of Active phase (25 months post-dose 1)	32	2623	0.6 (0.4; 0.8)	32	30	1310	1.1 (0.7; 1.6)	30	0.533	(0.31; 0.91)
	From Injection 1 to end of Year 3 (first year of Hospital Phase)	54	2460	2.2 (1.7; 2.9)	54	41	1230	3.3 (2.4; 4.5)	41	0.658	(0.43; 1.01)
	From Injection 1 to end of Year 4 (second year of Hospital Phase / SEP)	70	2378	2.9 (2.3; 3.7)	70	58	1190	4.9 (3.7; 6.3)	58	0.604	(0.42; 0.87)
	From Injection 1 to end of Year 5 (third year of Hospital Phase)	78	2321	3.4 (2.7; 4.2)	78	62	1163	5.3 (4.1; 6.8)	62	0.630	(0.45; 0.89)
	From Injection 1 to end of Year 6 (fourth year of Hospital Phase)	117	2274	5.1 (4.3; 6.1)	117	76	1140	6.7 (5.3; 8.3)	76	0.772	(0.57; 1.04)
CYD15	From Injection 1 to 28 days PD3 (1 year post-dose 1)	5	13916	<0.1 (0.0; 0.1)	5	15	6938	0.2 (0.1; 0.4)	15	0.166	(0.05; 0.48)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	12	13523	<0.1 (0.0; 0.1)	12	28	6748	0.4 (0.3; 0.6)	28	0.214	(0.10; 0.43)
	From Injection 1 to end of Active phase (25 months post-dose 1)	17	13720	<0.1 (0.0; 0.1)	17	43	6843	0.3 (0.2; 0.4)	43	0.197	(0.11; 0.35)
	From Injection 1 to end of Year 3 (first year of Hospital Phase)	33	13569	0.2 (0.2; 0.3)	33	58	6772	0.9 (0.7; 1.1)	58	0.284	(0.18; 0.44)
	From Injection 1 to end of Year 4 (second year of Hospital Phase / SEP)	39	13430	0.3 (0.2; 0.4)	39	67	6710	1.0 (0.8; 1.3)	67	0.291	(0.19; 0.44)

M: mean of number of subjects followed during the years included in the considered period. n Episodes: number of hospitalized virologically-confirmed dengue episodes.
Cases: number of subjects with at least one hospitalized virologically-confirmed dengue episode.
The annual Incidence rate= Cases among M * 100 converted in annual rate
Source: Appendix 1 – Clinical - Statistical Analysis, Table 2.67 and Table 1.4

Table 38: Incidence and Relative Risk of hospitalized clinically-severe virologically-confirmed dengue cases due to any serotype from Injection 3 to end of the Active Phase and from Injection 1 to end of each year of follow up – Efficacy Studies Integrated SafAS

		Dengue Group (N=23433)				Control Group (N=11690)				Relative Risk	
Study	Time period	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	RR	(95% CI)
CYD14	From Injection 1 to 28 days PD3 (1 year post-dose 1)	7	6849	0.1 (0.0; 0.2)	7	6	3423	0.2 (0.1; 0.4)	6	0.583	(0.17; 2.10)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	5	6813	<0.1 (0.0; 0.2)	5	13	3406	0.4 (0.2; 0.6)	13	0.192	(0.05; 0.57)
	From Injection 1 to end of Active phase (25 months post-dose 1)	12	6831	<0.1 (0.0; 0.1)	12	19	3415	0.3 (0.2; 0.4)	19	0.316	(0.14; 0.68)
	From Injection 1 to end of Year 3 (first year of Hospital Phase)	23	6814	0.3 (0.2; 0.5)	23	20	3405	0.6 (0.4; 0.9)	20	0.575	(0.30; 1.10)
	From Injection 1 to end of Year 4 (second year of Hospital Phase / SEP)	36	6799	0.5 (0.4; 0.7)	36	26	3398	0.8 (0.5; 1.1)	26	0.692	(0.41; 1.19)
CYD23/57	From Injection 1 to 28 days PD3 (1 year post-dose 1)	1	2668	<0.1 (0.0; 0.2)	1	0	1329	0.0 (0.0; 0.3)	0	NC	(NC)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	1	2578	<0.1 (0.0; 0.2)	1	2	1290	0.1 (0.0; 0.5)	2	0.250	(0.00; 4.81)
	From Injection 1 to end of Active phase (25 months post-dose 1)	2	2623	<0.1 (0.0; 0.1)	2	2	1310	<0.1 (0.0; 0.3)	2	0.499	(0.04; 6.89)
	From Injection 1 to end of Year 3 (first year of Hospital Surveillance)	6	2460	0.2 (0.1; 0.5)	6	2	1230	0.2 (0.0; 0.6)	2	1.500	(0.27; 15.19)
	From Injection 1 to end of Year 4 (second year of Hospital Surveillance)	7	2378	0.3 (0.1; 0.6)	7	4	1190	0.3 (0.1; 0.9)	4	0.876	(0.22; 4.08)
	From Injection 1 to end of Year 5 (third year of Hospital Surveillance)	8	2321	0.3 (0.1; 0.7)	8	5	1163	0.4 (0.1; 1.0)	5	0.802	(0.23; 3.12)
	From Injection 1 to end of Year 6 (fourth year of Hospital Surveillance)	10	2274	0.4 (0.2; 0.8)	10	5	1140	0.4 (0.1; 1.0)	5	1.003	(0.31; 3.74)
CYD15	From Injection 1 to 28 days PD3 (1 year post-dose 1)	0	13916	0.0 (0.0; 0.0)	0	5	6938	<0.1 (0.0; 0.2)	5	0.000	(0.00; 0.54)
	From Injection 3 to end of Active Phase (2 years post-dose 1)	0	13523	0.0 (0.0; 0.0)	0	6	6748	<0.1 (0.0; 0.2)	6	0.000	(0.00; 0.42)
	From Injection 1 to end of Active phase (25 months post-dose 1)	0	13720	0.0 (0.0; 0.0)	0	11	6843	<0.1 (0.0; 0.1)	11	0.000	(0.00; 0.20)
	From Injection 1 to end of Year 3 (first year of Hospital Phase)	3	13569	<0.1 (0.0; 0.1)	3	16	6772	0.2 (0.1; 0.4)	16	0.094	(0.02; 0.33)
	From Injection 1 to end of Year 4 (second year of Hospital Phase / SEP)	5	13430	<0.1 (0.0; 0.1)	5	16	6710	0.2 (0.1; 0.4)	16	0.156	(0.04; 0.45)

M: mean of number of subjects followed during the years included in the considered period.
Cases: number of subjects with at least one hospitalized virologically-confirmed dengue episode.
n Episodes: number of hospitalized clinically severe virologically-confirmed dengue episodes.
The annual Incidence rate= Cases among M * 100 converted in annual rate. NC: Not computable.
Source: Appendix 1 – Clinical - Statistical Analysis Table 2.66 and Table 1.1

All children fully recovered from hospitalized VDC cases including severe after supportive medical care. Considering both CYD14 and CYD15 studies, the clinical pattern of hospitalized SVCD cases during the first years of the Hospital Phase was similar to that observed during the Active Phase, with no increase in severity. Additional post-dengue disease viremia and immunological investigations also showed the absence of increase in severity of the dengue cases between the Active and the Hospital Phase.

At the time of cut-off date for Hospital Surveillance/Phase data presentation (September 2016), preliminary data collected in CYD57, CYD14 and CYD15 at year 2 of the long term follow-up show the same trend, i.e. a favourable benefit/risk ratio in overall subjects aged 9 years-old to 16 and an overall increase risk of hospitalized VCD including severe in subjects below 9 years of age.

During the Hospital Phase, hospitalized VCD and SVCD occurred with an increased incidence density in the endemic AP compared to the endemic LatAm regions, and there was an increase of hospitalized VCD and SVCD in the Dengue Group compared to the Control Group in the AP endemic regions from year 3 and beyond. More importantly, the incidence density of hospitalized VCD in dengue group increased dramatically during year 6 in the 9-11 years of age in CYD23/57 (no data available on the SVCD).

The Applicant was invited to discuss the most recent long-term follow-up data that reflect a difference between the 2 regions for hospitalized VCD and SVCD per same age group. The LTFU data with additional Year 3 HP/SEP data indicate that the risk for hospitalised and/or severe dengue may be decreasing over time. In the HP/SEP the RR is increased (1 or above) which may suggest waning of protective efficacy, but overall in the Entire study the RR is decreased (below 1) in particular in the older age group. In the youngest age group the RR is increased over the Entire study.

An important limitation of these LTFU data presented for the entire study population or for the different age categories is that they do not distinguish between seropositive and seronegative subjects. As has been demonstrated in the NS1 supplemental study, baseline serostatus rather than age seems to be affecting vaccine efficacy, see section 2.5.3 for data by baseline serostatus (NS1 analysis) and section 2.5.6 for discussion.

Laboratory findings

The laboratory parameters analysed were chemistry (creatinine, liver function tests and bilirubin) and haematology. They were selected based on changes in laboratory parameters observed in Phase I studies and on the biological abnormalities that can mimic dengue disease.

The pooled analysis of clinical laboratory data (676 subjects) showed that the majority of subjects had biological values within normal ranges both at baseline and after any CYD dengue vaccine injection. Biological safety abnormalities classified as Grade 3 were reported by low percentages of subjects (2.2% or less, depending on the parameter), and the most frequent ones were decreased haemoglobin and neutropenia. These biological safety abnormalities were transient and without medical consequences.

The biological safety profile of the CYD dengue vaccine was found to be overall similar to that of placebo or licensed vaccines.

Vaccine Viremia

Post-vaccination viremia was investigated in nonclinical and some clinical studies as an assessment of safety, but also as a measure of the bioavailability and replicative ability of the vaccine virus. For details see section 2.4.2. Briefly, vaccine viremia incidence was low (3.8% across pooled studies around D7 after the 1st injection) whatever the dengue immune status at baseline and the age group. No safety concerns were associated with vaccine viremia.

Within 28 days after the first and the second CYD dengue vaccine injections, 113 subjects and 106 subjects, respectively, experienced a febrile episode. Among them, only 1 subject had vaccine viremia, and no safety concerns were identified. A diagnosis of common cold was made by the Investigator. The acute blood sample was negative for dengue (i.e. not virologically-confirmed), but vaccine viremia, close to the LLOQ value, was however detected (CYD3, RT-PCR: 5.39 log10 GEq/mL).

No vaccine viremia was observed after injections 1 and 2 at timepoints other than those included in the pooled analysis i.e. timepoints < D5 (including before vaccination) and timepoints D18- D22. One subject had vaccine viremia after injection 3 (CYD06). No safety concerns were identified from this subject: the subject did not experience any solicited reactions, unsolicited non-serious AEs, or SAEs at the time of vaccine viremia.

In Phase I and early Phase II studies, vaccine viremia was assessed using both genomic amplification methods (RT-PCR assay) and virus culture (plaque assay [PA]). A total of 10 subjects had vaccine viraemia (8 post-Injection 1 and 2 post-Injection 3) that was quantified only by PA and not by RT-PCR and were not included in the pooled analysis. For all subjects, the level of vaccine viremia measured by PA was close to the LLOQ (1.6 log10 PFU/mL). None of the subjects experienced SAEs or AESIs at the time of vaccine viremia. Only 2 of these subjects experienced fever or Grade 3 AE (CYD04).

Several individuals experienced neurological symptoms such as malaise, myalgia and in singular cases high grade headache that have been addressed by the Investigators related to the vaccine, in 3 cases concomitantly with vaccine viremia, fever and neutrocytopenia (respectively from studies CYD10; CYD06; CYD11). The latter subject experienced Grade 3 headache without vaccine viremia at any timepoint 26 days after CYD dengue vaccine injection, which was assessed as related to the study vaccine by the Investigator.

Viremia was assessed in CYD14 and CYD15 studies in all subjects with VCD cases at any time after fever onset in acute sample collected during both the Active Phase and Hospital Phase, in order to further characterize the safety profile of these subjects. There was no increase of viremia levels from hospitalized including severe VCD cases between Active and Hospital Phases, or between CYD dengue vaccine

recipients and placebo recipients, indicating that CYD vaccination does not increase post-dengue disease viremia as compared to placebo recipients, in agreement with the absence of difference in dengue severity observed between the two groups.

Safety in special populations

Pregnancy and lactation

The use of the CYD dengue vaccine has not been studied in pregnant women since pregnancy was an exclusion criterion in all clinical studies with the CYD dengue vaccine and pregnancy testing was performed before each injection. The data are thus not sufficient to conclude on the absence of potential effects of Dengvaxia on pregnancy, embryo-foetal development, parturition and post-natal development. Animal studies did not indicate any direct or indirect harmful effects with respect to reproductive toxicity. As a precautionary measure, the vaccine is contraindicated during pregnancy and lactation, in line with other live vaccines. The SmPC further reflects that women of childbearing age should avoid becoming pregnant for 4 weeks after receiving any injection of Dengvaxia. 'Safety profile of inadvertent use in pregnant or lactating women' is included as a 'missing information' safety concern in the RMP. Close monitoring and long-term follow-up should be performed in this population.

The vaccine was inadvertently administered to female subjects who were not aware of their pregnancy or who became pregnant shortly after vaccination. For the purposes of the analysis, the pregnancies were classified in 3 categories: i) exposed to the study vaccine when the subject was pregnant (if the subject received the injection 7 days after her last menstrual period (LMP) or 7 days before the Estimated Date of Conception (EDC) (conservative risk window) or later during pregnancy); ii) exposed to the study vaccine when the subject was not yet pregnant (if the subject received the injection during the interval between 30 days before her LMP and 7 days after her LMP (which also corresponds to the period between 44 days and 7 days before EDC)); iii) unexposed (all other pregnancies).

As of 1st September 2015, a total of 404 pregnancies were reported in subjects who received the CYD dengue vaccine from completed studies or during the Active Phase of CYD14 and CYD15 studies (un-blinded data): 341 unexposed; 36 exposed, but not yet pregnant; 22 exposed and pregnant; 5 for which exposure could not be determined.

Most of the pregnancies were reported in CYD14 and CYD15 studies (in young adolescents), which were placebo controlled. Looking at the 2 subgroups classified as "exposed and pregnant" in the vaccinated and placebo groups, no difference between the 2 groups was observed in terms of abnormal pregnancy outcomes. In the Dengue Group, a total of 3 cases of abnormal pregnancy outcomes were reported, and in all cases important risk factors were identified.

As of 1st September 2015, a total of 601 pregnancies were reported during Hospital Surveillance/Phase of the efficacy studies. All pregnancies collected during Hospital Surveillance/Phase are unexposed (since they are far from conception), except for one lately reported pregnancy belonging to category "Exposed but not pregnant" that led to live birth. For the 587 pregnancies collected during Hospital Surveillance/Phase and classified as unexposed, the outcomes were: 434 live births, 91 ongoing, 46 abortions (spontaneous and unspecified), 10 stillbirths/death in utero, 3 unknown, 2 elective terminations of pregnancy, and 1 ectopic pregnancy. For 13 pregnancies, no information was provided regarding the timing of their exposure to the vaccine. The outcomes of these pregnancies were live birth (4 cases), spontaneous abortion (5 cases), the pregnancy was still ongoing in 4 cases at the time of assessment.

In conclusion, no safety signals identified from the review of these abnormal pregnancy outcomes.

There is no data on lactation.

Babies born from women vaccinated with CYD dengue vaccine during pregnancy

SAEs that occurred after 20 weeks of gestation in babies born from pregnant subjects were reported as "baby cases". In the event of abnormal pregnancy outcome, such as stillbirth, 1 case was created for the mother and 1 for the baby.

Among the baby cases not linked with abnormal pregnancy outcomes, no additional cases were reported from the pregnancies where the mother was pregnant at the time of the CYD dengue vaccine or placebo injection.

Five baby cases, all in CYD15 study, were reported for pregnancies exposed to the CYD dengue vaccine when the mother was not yet pregnant. These events were mainly infections (left unilateral conjunctivitis and viral pneumonia, and 2 cases of neonatal sepsis) or linked to prematurity, all in CYD15.

In the Placebo Group, there was 1 case of neonatal sepsis. No cases of congenital abnormalities were reported in either category of exposed pregnancies.

One case of stillbirth was reported in a 16-year old female subject in CYD15, who received two doses of the CYD dengue vaccine. She was exposed to vaccine before pregnancy (last vaccine dose received on 10 January 2012 and LMP was 18 January 2012). The event of stillbirth was reported by the investigator as unrelated to the investigational vaccine.

No data is available for infants born from women vaccinated with CYD before pregnancy.

Dengue Status at Baseline

The clinical safety profile was similar in terms of type of ARs in subjects who were dengue non-immune and immune at baseline with regard to non-Dengue AE, but the ARs were observed at lower frequencies in dengue seropositive subjects especially in adults. The impact of dengue status at baseline on SVCD occurring during the Active Phase in the efficacy studies was confirmed in post-hoc analysis (see Supplemental NS1 study).

Other Status at Baseline

The impact of FV, JE and YF status at baseline was limited to trends of differences in incidence of occasional safety parameters, the majority of safety parameters had similar incidence in both YF and JE non-immune and immune subjects. The impact of YF and JE status at baseline on SVCD occurring during the Active Phase in the efficacy studies could not be assessed due to sample size limitation.

Safety related to drug-drug interactions and other interactions

There are currently no data available from co-administration studies within the age range of the indication from endemic settings.

Three small co-administration studies were previously conducted in toddlers with YF, DTaP-IPV/Hib, and MMR vaccines. These studies were undertaken in Colombia/Peru, Mexico, and the Philippines, respectively. In adults, one study has been conducted in the US with YF, but with a different CYD schedule than the one proposed for authorisation. From these small studies it was concluded that there were no safety concerns (data were comparable when vaccines were co-administered or given alone), and that the immunogenicity profile was satisfactory both for CYD and for co-administered vaccines. The one exception to this was a lower response to serotype 4 in the study in US adults. In the labelling, there are no data with co-administration included because the toddler age group and non-endemic populations are outside the requested indication.

Co-administration therefore is not recommended at this stage.

Studies are currently ongoing on co-administration of CYD dengue vaccine with HPV vaccine or booster Tdap/Tdap-IPV in the age range of the indication (see RMP).

Discontinuation due to adverse events

AEs and SAEs leading to discontinuation were collected through the studies in all the trials.

Group 9 to 17 years of age: A total of 102 subjects discontinued due to a non-serious AE or SAE; 64 (0.3%) in the Dengue group and 38 (0.4%) in the Placebo group. Non-serious AE leading to discontinuation were reported in 13 out of 3,067 subjects from the Dengue group. Asthmatic crisis and urticaria were both Grade 3 and were assessed as related to Dengue vaccine. They were considered as AESIs. In the Dengue group SAEs leading to discontinuation were reported in 32 out of 19,120 subjects, of which 4 non fatale SAEs were assessed as related to the Vaccine (see SAEs section).

Adults: In the main studies SafAS and RS (1306 subjects) a total of 19 subjects discontinued due to a non-serious AE or a SAE, i.e. 18 (1.2%) in the Dengue Group and 1 (0.5%) in the Placebo Group. In the Dengue Group, non-serious AEs leading to study discontinuation after any injection were reported in 7 subjects. Among these, there was one AEs reported as Grade 3: periorbital infection (in 1 subject each), which was assessed as related to the study vaccine by the Investigator. Two other AEs were assessed as related to the study vaccine by the Investigator: viral upper respiratory tract infection, and spinal osteoarthritis, reported each in 1 subject. In the Placebo Group, no AE led to study discontinuation. In the Dengue Group, SAEs leading to study discontinuation after any injection were reported in 9 subjects. Most SAEs were isolated in terms of nature and were reported as isolated events. Three subjects experienced 1 SAE that was assessed as related to the study vaccine by the Investigator (polymyalgia rheumatica, blighted ovum, and headache).

Group 6 to 11 years of age: A total of 50 subjects discontinued due to a non-serious AE or SAE; 30 (0.2%) in the Dengue group and 20 (0.3%) in the Placebo group. Of these, one case of Grade 2 urticaria and 4 cases of SAEs were assessed as related to the Vaccine by the investigator and sponsor (see previous section on SAEs).

Group 2 to 5 years of age: a total of 10 subjects discontinued due to a non-serious AE or SAE; 5 (0.2%) in the Dengue group and 5 (0.4%) in the Placebo group. Of these, one case of Grade 3 hypersensivity was assessed as related to Vaccine and considered as an AESI.

Post marketing experience

The CYD vaccine has not been implemented in any country-wide programme to date but it has been introduced in two subnational programs in the Philippines and Brazil targeting in total about one million individuals. It is otherwise available on the private market in at least 10 countries where there is a marketing authorization.

In March-April 2016, The Philippines launched a school-based dengue immunization program in more than 700.000 students of 9-11 years of age. A total of 10 fatal cases due to dengue disease were reported to have occurred after vaccination from worldwide post-marketing setting (including public programs in the Philippines and the Parana state of Brazil).

Upon request, the Applicant submitted the PBRER covering the period 08/12/2017 to 07/06/2018. Of note this PBRER summarises the post-marketing experience in countries wherein CYD vaccine is indicated based on endemicity, i.e. without discriminating subjects with previous dengue infection(s). The signal on the increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously

infected by dengue virus was validated and analysed during the previous reporting period (08-June-2017 through 07-December-2017). Due to the difficulties to collect relevant post-marketing data on hospitalised and severe dengue cases in vaccinees in the Philippines, the assessment of most fatal cases reported in the PBRER was incomplete. During this period until June 2018, a total of 49 fatal cases were reported. Fifteen out of the total fatal cases were dengue cases with fatal outcome and their evaluation was ongoing at the time of this application. The WHO is closely monitoring and investigating the serious severe and fatal cases in collaboration with the Filipino authorities. The WHO Global Advisory Committee on Vaccine Safety (GACVS) published a report on 20 July 2018 discussing the safety of dengue vaccine in the Philippines, among other topics (WER, No. 29/30, 2018, 93, 389-396).

Overall, the available information is limited and does not provide safety risk by serostatus, hence it is not possible to draw any conclusion at this stage. The Applicant will monitor and report in PSURs any new data on dengue severe disease.

During the review of marketing data (18 September 2016), one safety signal of allergic (including anaphylactic) reactions has been identified. Based on the analysis of these reactions, they have been identified as "important identified risk" in RMP and listed in the SmPC. No other signal has been identified in the review of marketing data.

2.6.1. Discussion on clinical safety

A total of 27,643 subjects aged 9 months through 60 years included in the studies used in the pooled/integrated analysis received at least 1 injection of the final formulation. The pooled database allowed for the detection of very common, common, and uncommon AEs and SAEs with an incidence ≥ 0.1% with a probability of at least 95%. This level of precision was in accordance with WHO guidelines. In the 16 studies using the final vaccination schedule (3 injections administered at 6-month intervals), 20,667 subjects aged 9 years through 60 years received at least 1 injection of the CYD dengue vaccine: 1547 adults 18-60 years old, and 19,120 children and adolescents aged 9-17 years.

Reactogenicity and non-Dengue related safety

The follow-up period for reactogenicity data and SAEs data was 6 months post-dose 3.

Adverse reactions were collected within 28 days after any injectionon a reactogenicity subset of 1547 adults and 3068 children (subjects from 9 to 60 years of age) from the main studies. The most frequently reported reactions (between 5% and 54% of subjects) whatever the age group were headache, injection site pain, malaise, myalgia, asthenia, and fever. In subjects 9 to 45 years of age, the most frequently reported reactions whatever the dengue serostatus prior to vaccination, were headache (54%), injection site pain (49%), malaise (44%), myalgia (43%), asthenia (34%), and fever (16%). Adverse reactions occurred within 3 days following vaccination except fever which appears within 14 days after the injection. The adverse reactions were usually mild to moderate in severity and of short duration (0 to 3 days). Systemic adverse reactions tended to be less frequent after the second and third injections of Dengvaxia as compared to the first injection.

Less than 6% (38 subjects out of 683) subjects had vaccine viremia after administration of the CYD dengue vaccine. In each case, vaccine viremia recorded was low and no safety concerns were observed in these subjects. There was no apparent difference in viraemia levels or cytokine profiles, including by age group, which has been argued to be counter to an immune enhancement hypothesis.

The impact of age group, gender, and region was limited to trends toward differences in incidences of safety parameters for the >9 years of age. As the trends were generally also observed in the Placebo

Group, an impact of reporting practices in the various populations was suspected. Overall, the same adverse reactions but at lower frequencies were observed in dengue seropositive subjects.

Serious Adverse Events within 28 days after any injection were reported in approximately 1% of subjects (between 0.6% and 1.8% depending on the age group), and were mainly diseases, infections or injuries commonly reported in these age groups, and no cluster in terms of nature and frequency was observed. A total of 4 neurological disorder SAEs within 30 days were assessed as related to the study vaccine by the Investigator (headache, tension headache, acute polyneuropathy, and ADEM) in addition to convulsion that was assessed as related to the study vaccine by the Sponsor only. For the ADEM case, acute polyneuropathy and convulsion, no vaccine viruses were isolated from the subjects.

Occurrence of SAEs, including serious AESIs was recorded in all subjects throughout the entire studies. During the long-term safety follow-up, no SAEs assessed as related to the study vaccine were reported from Month 6 onwards after the last injection in the Dengue Group. No deaths were linked to dengue cases.

The analysis of AESIs showed no concerns in terms of allergic reactions, as no anaphylactic reactions were retrieved by the SMQ algorithm in the Dengue Group, and the proportions of subjects who reported non-serious potential allergic reactions were low and similar between the Dengue Group and the Placebo Group. In addition, very few potential allergic reactions were rated as Grade 3 or serious. However, as anaphylactic reactions were observed during the post-marketing mass vaccination campaign in the Philippines, this AE is now included in section 4.8 of the SmPC. No events of viscerotropic or neurotropic disease were observed after administration of the CYD dengue vaccine was observed in any studies.

The majority of uncommon adverse reactions to be included in the SmPC were reported more frequently, or exclusively, in adults. Given the small number of adults that have received the dengue vaccine it is conceivable that the frequency of some uncommon adverse events is being underestimated and/or other potential uncommon adverse reactions have not been identified for this age group. This remains a limitation but safety surveillance data will be collected and assessed post-authorisation.

Data of safety related to interactions to other vaccines are very limited. More data would be needed to assess safety of Dengue vaccine when administered sequentially or co-administered with other vaccines.

Discontinuation due to AEs were comparable between the adult Dengue group (1.7%) and other age groups (<1%).

Hospitalized VCD and SVCD cases during Active and Hospital Phase

Long-term follow-up was defined as the period from Month 6 after the last injection onward for SAEs and from 1 year after the last injection onward for dengue cases. As of December 2017, the following data from on-going long-term follow-up were available from the efficacy studies:

- CYD57: full data from the 4 years of Hospital Phase (Hospital Phase Year 1 to Year 4, i.e., 2 to 5 years after the last injection in CYD23).
- CYD14 and CYD15: full data from the first 3 years of Hospital Phase (Hospital Phase Year 1, Year 2 and Year 3, i.e., 2, 3 and 4 years after the last injection).
- NS1 Supplemental analysis: until March 2017, i.e. 4 years of the Hospital Phase for CYD57; a minimum of 3 years and 3 months of Hospital Phase/Surveillance Expansion Period (SEP) surveillance for CYD14; and a minimum of 3 years of Hospital Phase/SEP surveillance for CYD15. Additonal LTFU data was submitted and assessed during the procedure and the Applicant commits to provide the final CSRs of CYD14 and CYD15, when available as indicated in the RMP.

Year 1, Year 2 and Year 3 safety long-term follow-up results from the pooled analysis of CYD14, CYD23/CYD57 and CYD15 showed that, in the subjects 9 years and above, there was a decreased risk of hospitalized VCD cases in the Dengue Group (cumulative RR of 0.535), and no evidence of excess of clinically SVCD cases in the Dengue Group compared to the Control Group (cumulative RR of 0.874). In subjects aged below 9 years, there was a trend toward an increased risk of hospitalized VCD (1.146) and SVCD (1.330). CYD57 which provided 4 year of long-term follow-up data, confirmed that, although the RR fluctuated over time, there was a decreased risk of hospitalized VCD in the Dengue Group (cumulative RR of 0.511), and no evidence of excess of hospitalized SVCD cases in the Dengue Group compared to the Control Group (cumulative RR of 1.023).The clinical pattern of hospitalized SVCD cases during the 3 first years of the Hospital Phase was similar to that observed during the Active Phase, with no increase in severity. Additional post-dengue disease viremia and immunological investigations also showed the absence of increase in severity of the dengue cases between the Active and the Hospital Phase. Therefore, there is no evidence, clinically, immunologically or virologically, that the disease in the Dengue Group is more severe to that observed with wild-type infection in the Control Group.

The clinical severity in the vaccinated seronegative group was similar to that of severe cases in the unvaccinated seropositive group except for plasma leakage and thrombocytopenia. In the clinical trials for those aged 9 years and above, the cases of severe dengue that occurred in initially seronegative vaccine recipients were categorized by the company as Dengue Hemorrhagic Fever Grades I and II and did not lead to shock, severe bleeding or death. All of the patients with dengue illnesses recovered.

Although, a decreased risk of hospitalized and clinically SVCD was observed in subjects 9 years of age and older over the entire duration of the study and during the Hospital Phase, imbalance in the occurrence of hospitalized dengue cases in the youngest vaccine recipients in CYD14 (subjects aged 2 to 5 years at enrolment) during the first year of the Hospital Phase was observed. This observation could be explained by 3 main interconnected hypotheses:

1. Potential lower quality cross-reactive responses may be more prone to waning. This waning would be more significant in younger children more likely to be seronegative at the time of vaccination (whose vaccine elicited PRNT50 set-point is lower).
2. Increased risk in seronegative subjects due to vaccination acting as a primary infection with condensed enrolment clustering vaccinees compared to placebo recipients who would be primed naturally over a longer period. Continued observation may show equalization over time.
3. Age per se may be important. Younger aged children may have less developed micro-vascular physiology and partially immature immune responses

In order to identify a potential impact of the baseline dengue serostatus on the safety and efficacy of CYD dengue vaccine, the 3 efficacy trials were re-analysed using a novel assay (anti-NS1 ELISA assay) to infer dengue serostatus and a case-cohort study design. This NS1 supplemental study analysis found that dengue serostatus at baseline modified the risk of hospitalized dengue and severe dengue after vaccination. In subjects dengue seropositive prior to vaccination, a decreased risk against hospitalized and severe dengue over the long-term follow-up period was observed following vaccination in subjects 9 to 16 years of age. In seronegative subjects 9 years of age and older, a trend towards an increased risk of dengue hospitalization and severe dengue was observed with the onset of risk mainly observed during the 3rd year after first vaccination. The clinical profile of severe cases in seronegative subjects was comparable between the vaccine group and control group.

Adults

The Safety profile from 1,547 subjects 18 to 60 years was analysed. No SVCD were reported in the non-efficacy Phase I-II CYD05, CYD22 and CYD28 studies concerning the adult population 18-45 years of age. These data concerned approximatively 550 exposed to Dengvaxia on 4 years post-dose 3 long-term follow-up, which are limited to conclude on especially on AESIs such as VCD hospitalized and Severe VCD hospitalized. However, the safety profile of the CYD dengue vaccine in adults aged 18-60 years was also similar to that observed in the other age groups, with less fever than in children and more non-serious unsolicited adverse reactions reported (11.6%) than in adolescents (2.0%) and children (2.5%) profiles. I addition, several new trials are ongoing or will be starting soon, as part of the RMP that will complement the information on safety of the vaccine in subjects above 18.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

Additional expert consultations

See section 2.5.6.

2.6.2. Conclusions on clinical safety

CYD vaccine elicited the common adverse events associated with vaccination such as pyrexia, and injection site pain and injection site reactions. The frequency of AE related to reactogenicity was acceptable. Deaths were reported with a similar frequency in both Dengue and Control Groups. No deaths were considered related to the study vaccine in any of the studies. Post-marketing pharmacovigilance data in the Philippines identified a safety signal, which was included in the RMP as important identified risk: Allergic (including anaphylactic) reactions. The safety profile of the CYD dengue vaccine in relation to non-Dengue adverse events is considered acceptable in the light of expected benefit.

Long-term follow-up (LTFU), defined as Surveillance/Hospital Phase studies, aimed at detecting hospitalized virologically confirmed cases (VCD) and severe dengue cases (SVCD) were performed. In all studies, a reduced risk of hospitalization due to dengue was confirmed in subjects ≥9 years during the entire Hospital Phase: the 3-year cumulative RR in CYD14 and CYD15 was 0.666 and 0.517, respectively; and despite fluctuating RRs over time, the 4-year cumulative RR in CYD57 was 0.511.

Data from year 1 of the Hospital Phase (PD3 period) for the age group 2-5 years of CYD14, showed that there were 15 cases of hospitalized dengue in the vaccinated group and 1 in the control (randomization ratio 2:1), which resulted in a RR of 7.454 (1.15-313.80). This population is outside of the age range filed by the Applicant for approval, nevertheless raised concerns. Based on supplementary post-hoc analysis of up to 6 years of follow up from the first injection in three efficacy studies, such increased risk of hospitalisation for dengue including clinically severe dengue was confirmed to be limited to baseline dengue seronegative vaccinees (i.e. with no previous dengue infection), as measured by PRNT50, regardless of age. Over a period of 5 years after the first injection, in subjects 9-16 YOA with no previous dengue infection, the risk of severe dengue is increased by 2.43 fold (95% CI: 0.47; 12.56) in vaccinees as compared to control subjects in the same age group. In the clinical trials for those aged 9 years and above, the cases of severe dengue that occurred in initially seronegative vaccine recipients were categorized by the company as Dengue Hemorrhagic Fever Grades I and II and did not lead to shock, severe bleeding or death. All of the patients with dengue illnesses in the trial recovered.

The increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously infected by dengue virus is therefore included in the RMP as important identified risk and it will be followed up post-authorisation. The mechanism that leads the observed increased risk of dengue in seronegative subjects at baseline is unknown. In subjects 9 years of age or older, it was estimated that during a 5 year

follow-up about 5 additional hospitalized dengue cases or 2 additional severe dengue cases per 1000 vaccinees with no previous dengue infection could occur following vaccination. Estimates from the long-term analysis suggest the onset of increased risk was mainly during the 3rd year following the first injection.

This increased risk was not observed in individuals who have been previously infected by dengue virus, where it was estimated that 15 hospitalized dengue cases or 4 severe dengue cases could be prevented per 1000 vaccinees with previous dengue infection during 5 years of follow up from the first injection. However, these figures may not be extrapolated to other regions with different seroprevalence and epidemiological situations as compared to the regions where the trials were conducted.

There is limited safety data in adults. In immunological and safety terms, subjects from 9 to 45 years of age are comparable to subjects 9-16 years of age, and therefore since no excess risk is observed for seropositive subjects 9 to 16 years old it is reasonable to assume that a similar situation would apply to 16 to 45 year-olds. More data in adults 18-45YOA will be collected through clinical studies assessing the effect of a booster dose (CYD63, CYD64), different administration schedules (CYD65) and the concomitant administration of another vaccine (CYD66). As per the risk management plan, additional safety data will be collected in adults through a long term PASS (DNG15) as well as through effectiveness studies planned to be conducted during 5 years in Mexico, Brazil, Malaysia and the Philippines.

The risk of severe dengue in infants born to vaccinated seronegative women remains hypothetical. As for situation of natural maternal dengue infection, pre-existing dengue antibodies may not be the cause or the only reason for such disease severity in infants. Other factors such as an immature immune system, immunological naivety, predisposition to dehydration and shock and a leaky circulatory system may be greatly contributing to the clinical profile of severe dengue in infants. The theoretical risk of severe dengue due to vaccination of non-immune dengue women in reproductive age should be very limited since vaccination of individuals seronegative at the time of vaccination is not recommended. There is insufficient evidence to consider this theoretical risk a safety concern for the vaccine; however, pregnancy exposure cases will continue to be closely monitored in the PBRER; in addition, hospitalized dengue cases in infants up to 1 year after birth will be collected in planned pregnancy registry study DNG16.

As there is limited data in individuals residing in non-endemic countries and no data in individual residing in non-endemic countries and traveling to endemic countries, the CYD dengue vaccine should not be used for travellers even if seropositive nor in the context of dengue outbreak in non-endemic regions.

No specific studies have been performed on concomitant administration of Dengvaxia with any other medicinal product(s) in individuals 9 to 45 years of age living in endemic areas. This is considered as missing information and listed as such among the safety concerns in the RMP. More data will be generated post-authorisation.

A risk of severe dengue disease due to waning of vaccine-protection over time is considered an important potential risk in the RMP and it will be followed up in post-marketing.

No data is available in immunosuppressed subjects. Since this is a live vaccine, the use in this population is contraindicated. However some data will be generated post-authorisation.

Because Dengvaxia is based on yellow fever vaccine construct, there might be a potential risk of yellow fever vaccine-associated viscerotropic and neurotropic disease, which will be followed up via routine safety monitoring in the post-authorisation phase as well as in a cohort event monitoring study.

The CHMP considers that the following measures foreseen in the RMP will help to address the remaining uncertainties or missing information related to safety:

- DNG15 Cohort Event Monitoring (CEM): the primary objective is to evaluate the safety profile of CYD dengue vaccine when used in the real-world immunization setting. The secondary objective is to describe, by country, the population vaccinated with CYD dengue vaccine in the real-world immunization setting, according to age, gender, number of doses received and interval between doses, comorbidities including immunosuppression, co-vaccination, history of recent vaccination, history of dengue, use in pregnancy or lactation, and misuse.
- DNG16 Pregnancy registry: The primary objective is to collect maternal, foetal, and infant outcomes of potential exposure to dengue vaccine during pregnancy. Hospitalized dengue cases in infants will be collected up to 1 year after birth.

2.7. Risk Management Plan

Safety concerns

Important Identified Risks	• Allergic Reactions (including anaphylactic) reactions • Increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously infected by dengue virus
Important Potential Risks	• YF vaccine-associated viscerotropic disease (YEL-AVD) • YF vaccine-associated neurotropic disease (YEL-AND) • Risk of severe dengue disease due to waning protection against dengue disease over time
Missing Information	• Safety in immunocompromised subjects (including subjects with congenital or acquired immune deficiency, or with Human Immunodeficiency Virus (HIV) infection with impaired immune function) • Safety profile of inadvertent use in pregnant or lactating women • Co-administration of CYD dengue vaccine with HPV vaccine or booster dose of Tdap vaccine

Pharmacovigilance plan

Study/activity Type, title and category	Objectives	Safety concerns addressed	Status	Planned date for submission of final reports
DNG15 Cohort Event Monitoring (CEM) study Category 3	The primary objective is to evaluate the safety profile of CYD dengue vaccine when used in the real-world immunization setting. The secondary objective is to describe, by country, the population vaccinated with CYD dengue vaccine in the real-world immunization setting, according to age, gender, number of doses received and interval between doses, comorbidities including immunosuppression, co-vaccination, history of recent vaccination, history of dengue, use in pregnancy or lactation, and misuse.	Safety in real-life use Exposure during pregnancy and lactation Allergic/anaphylactic reactions Viscerotropic and neurotropic diseases	Ongoing	31 December 2025
DNG16 Pregnancy registry Category 3	The primary objective is to collect maternal, fetal, and infant outcomes of potential exposure to dengue vaccine during pregnancy. Hospitalized dengue cases in infants will be collected up to 1 year after birth.	Exposure during pregnancy	Planned	31 December 2023

Study/activity Type, title and category	Objectives	Safety concerns addressed	Status	Planned date for submission of final reports
CYD52, CYD53, CYD69, CYD70 and DNG10042 Effectiveness studies Category 3	The objective is to assess the vaccine effectiveness at the community level, as well as effectiveness at reducing frequency of hospitalization and severe forms of dengue disease. In addition, these studies will provide a platform to identify a potential increase in disease severity and a potential waning of protection over time.	Waning of protection over time.	Planned	31 December 2020 for DNG10042 31 December 2023 for CYD69 31 December 2025 for CYD52, CYD53, and CYD70
CYD14 and CYD15 Efficacy studies-amendments to long-term follow-up Category 3	The objective of the amendments in CYD14 and CYD15 is to capture the full range of dengue disease in the study population prospectively (i.e. return to active detection of all symptomatic dengue cases). Long-term safety monitoring.	Long term efficacy Increased risk of severe and hospitalized dengue upon vaccination in individuals not previously infected by dengue virus Waning of protection over time	Ongoing	31 March 2019 for CYD14 31 March 2019 for CYD15
CYD63, CYD64, and CYD65 Booster studies Category 3	The studies evaluate the safety and immunogenicity of a booster dose of dengue vaccine administered in a subset of subjects who received third dose of dengue vaccine 4-5 years before, in Phase II studies (CYD63 and CYD64). Immunogenicity and Safety of Tetravalent Dengue Vaccine Given in 1-, 2-, or 3-Dose Schedules Followed by a Single Booster (CYD65)	Waning of protection over time Need for booster	Ongoing	31 December 2019 for CYD63 and CYD64 31 March 2021 for CYD65
CYD50 Exposure in HIV+ population Category 3	Immunogenicity and safety in HIV+ adult subjects with stable clinical condition under antiretroviral therapy.	Exposure in immunocompromised population	Planned	30 June 2022
CYD66, CYD67, and CYD71: Co-administration studies Category 3	The studies evaluate the safety and immunogenicity of co-administration of CYD dengue vaccine with other vaccines: booster dose of Tdap (CYD66), HPV Vaccine (CYD67 and CYD71).	Co-administration with Tdap, HPV vaccines	Ongoing	31 December 2020
Cross sectional survey to evaluate vaccinator's knowledge and understanding Category 3	A cross sectional survey to evaluate vaccinator's knowledge and understanding of the restricted indication to only individuals previously infected will be used to measure the effectiveness of risk minimisation measures (HCP guide) in Europe.	Increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously infected by dengue virus	Planned	31 December 2022

Risk minimisation measures

Safety concern	Routine risk minimization measures	Additional risk minimization measures
Allergic/anaphylactic reaction	In the SmPC/PI: In the Section "Contraindications", it is stated that the CYD dengue vaccine must not be administered to individuals with a history of hypersensitivity to any component of the vaccine or after prior administration of the vaccine or of a vaccine containing the same components. In the Section "Special warnings and precautions for use", it is stated that appropriate medical treatment and supervision must always be readily available in the event of an anaphylactic reaction following administration of the vaccine. Allergic (including anaphylactic) reactions are considered as listed events.	None
Increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously infected by dengue virus	In the SmPC/PI: The section Indication has been updated to ensure that vaccination is given only to individuals in whom B/R was demonstrated to be positive: "Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with documented prior dengue virus infection and living in endemic areas." And in the Section "Special warnings and precautions for use", it is stated that Dengvaxia is not indicated For individuals who have not been previously infected by dengue virus. It is also states in this section that vaccination is not recommended for individuals living in non-endemic areas and traveling to endemic areas.	HCP guide
YEL-AVD and YEL-AND	None	None
Risk of severe dengue disease due to waning protection against dengue disease over time	In the SmPC/PI: In the Section "Special warnings and precautions for use", it is stated that it is recommended to continue personal protection measures against mosquito bites after vaccination.	None
Absence of Safety Data in immunocompromised subjects (including subjects with congenital or acquired immune deficiency, or with HIV infection with impaired immune function)	In the SmPC/PI: In the Section "Contraindications , it is stated that the CYD dengue vaccine must not be administered to individuals with congenital or acquired cell-mediated immune deficiency, including immunosuppressive therapies such as chemotherapy or high doses of systemic corticosteroids within 4 weeks prior to vaccination. It is also stated in this section that CYD dengue vaccine must not be administered to individuals with symptomatic HIV infection or with asymptomatic HIV infection when accompanied by evidence of impaired immune function.	None
Inadvertent use in pregnant or lactating women	In the SmPC/PI: In the Sections "Contraindications", "Fertility, Pregnancy and Lactation", it is stated that the CYD dengue vaccine must not be administered in case of pregnancy/ lactation.	None
Absence of data on Co-administration of CYD dengue vaccine with HPV vaccine or booster dose of Tdap vaccine	None	None

The Applicant proposes a Guide for Health Care Professionals (HCP) as an additional risk minimization measure(s) to reinforce the prescriber's awareness about the increased risk of severe and hospitalized

dengue upon vaccination in individuals not previously infected by dengue virus. The guide will provide label information on the risk for individuals not previously infected by dengue virus and guidance on how to assess the prior dengue infection in these individuals before vaccinating.

It is considered important that this Guide contains sufficiently detailed and accurate information on the required characteristics for serotests assessing previous dengue infection. The necessity of achieving high specificity and positive predictive values should be emphasized, as well as the need to use tests with no cross-reactivity with flaviviruses circulating in the area or vaccination against other flaviviruses. It is considered that high specificity is required whatever the epidemiological context because of inevitable uncertainties on seroprevalence levels, and on pre-test probabilities (highly locally variable, variable over time, variable according to demographic characteristics).

The Guide should refer to local guidance published by the public health authorities responsible for the EU area to be vaccinated, and emphasise that only tests recommended by this PH authorities should be used.

Conclusion

The CHMP and PRAC considered that the risk management plan version 5.0 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the Applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The Applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 08/12/2015. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The Applicant declared that dengue tetravalent vaccine (live, attenuated) has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers that the active substances of the Dengvaxia vaccine, i.e. live attenuated chimeric dengue virus (CYD) serotypes 1-2-3-4 to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the Applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Quick Response (QR) code

A request to include a QR code in the labelling for the purpose of providing to patients information on dengue disease and on vaccination has been submitted by the Applicant and has been found acceptable.

The following elements have been agreed to be provided through a QR code: Information on Dengue, a path to the on-line product information on EMA website, recommendations during and post vaccination, and what to do in case of any adverse event.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Dengvaxia (dengue tetravalent vaccine (live, attenuated)) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Dengue is an acute, systemic viral infection caused by 4 closely related but antigenically distinct virus serotypes (1, 2, 3, and 4) transmitted primarily by the *Aedes aegypti* mosquito. Dengue is the most common mosquito-borne viral disease in humans, spreading globally during the past 30 years as a result of changes in human ecology. The infection may be asymptomatic, cause flu-like illness, and can in a small proportion of patients develop into a potentially lethal complication called severe dengue (including dengue haemorrhagic fever [DHF]/dengue shock syndrome [DSS]).

The progression to a severe form occurs via a pathophysiological host response to infection leading to vascular permeability, plasma leakage, microvascular bleeding and reduced functioning of the coagulation cascade. This is defined by one or more of the following: (i) plasma leakage that may lead to shock and/or fluid accumulation (DSS), and/or (ii) severe bleeding, and/or (iii) severe organ impairment (liver, CNS, heart).These severe manifestations occur infrequently, in <1% of infections, and involve bleeding of multiple organs and fluid accumulation within body cavities. Improvement of dengue case detection, identification of warning signs and early initiation of appropriate treatment have decreased the severity (including mortality) of dengue disease.

Infection by each serotype is considered to induce serotype-specific immunity.

In endemic areas, the entire population is at risk of dengue infection. The disease affects all age groups. The age distribution of infected individuals varies between countries and no clear pattern of populations at risk has been identified. Additionally, the population at highest risk can shift over time. The prevalence of each serotype fluctuates over time, as does the genetic diversity within each serotype. The four dengue virus serotypes are genetically diverse and share limited aminoacid identity (around 60-75%). Genetic variations between serotypes and clades may be important determinants of differential viral fitness, virulence and epidemic potential

3.1.2. Available therapies and unmet medical need

There is no specific treatment for dengue disease. The management of dengue disease is supportive, with rest, control of fever and pain with antipyretics/analgesics, and adequate fluid intake. Supportive intensive care and fluid management are the mainstays of therapy for severe disease.

The global incidence of dengue has grown dramatically in recent decades and half of the world's population is now considered at risk of infection by the dengue viruses. Worldwide, an estimated 390 million dengue infections occur every year, of which around 100 million are associated with clinical manifestation of dengue. Around 500,000 hospitalizations are reported each year, and around 20,000 cases would result in death.

The prevention of dengue used to be by vector control, interruption of human-vector contact by personal protection measures, and community engagement focused on awareness, education, mobilization and communication to sustain control measures. None of these either alone or in combination has had a significant impact on incidence of dengue disease. Hence, there was an urgent need to develop a safe and effective vaccine against the four serotypes of dengue virus to protect people in endemic countries, as recognized by WHO.

In addition to children, adults are also at risk of dengue disease. This is the key reason for seeking an indication in both children and adults.

3.1.3. Main clinical studies

The CDP for dengue vaccine involved the assessment of several vaccine formulations and schedules in a diverse population. As of December 2017, the CDP included 31 clinical studies, completed (22) or on-going (9): 5 Phase I, 17 Phase II and 9 Phase III, for a total of 41,000 subjects enrolled. Among these subjects, 20,974 subjects were in the target age indication (9 to 45 years of age), of which 1741 adults aged 18 to 45 years receiving at least one dose.

Three pivotal efficacy and safety studies were conducted: CYD14 and CYD15 (studies in multiple countries in AP [in subjects from 2 to 14 years] and LatAm [in subjects aged 9 to 16 years], respectively) following the Phase IIb proof of concept study in Thailand in subjects aged 4 to 11 years (CYD23 and its long term follow up CYD57). Each individual Phase III efficacy study was sufficiently powered to demonstrate significant efficacy of the CYD dengue vaccine in preventing the occurrence of VCD cases due to any serotype after 3 injections. Immune response to the vaccine was also evaluated in a subset of the population as well as its relationship to the observed efficacy.

3.2. Favourable effects

In subjects 9-16YOA, VE was demonstrated in both efficacy studies with 67.8% and 64.7% in CYD14 and CYD15 respectively (meta-analysis showing overall efficacy of 65.6% in the 9 to 16 years population) against any serotype after at least 1 injection of the CYD dengue vaccine during the first 25 months of the studies (active phase). The primary endpoint in each study was met, demonstrating efficacy against VCD cases post-injection 3 due to any serotype with the lowest lower bound of the 95% CI of 57.7% in CYD14.

Significant VE was also observed in preventing the occurrence of VCD case due to each serotype after at least 1 injection of the CYD dengue vaccine. Efficacy varied according to the serotype: moderate efficacy was observed for serotypes 1 and 2 (58.4% and 47.1% respectively from the meta-analysis) and high efficacy was observed for serotypes 3 and 4 (73.6% and 83.2%). This finding was consistent across the regions evaluated. The lower efficacy against serotype 2 might be influenced by a bias due to the high proportion of seronegative subjects exposed to dengue serotype 2.

During the 25-month of the Active Phase observation period of the Phase III efficacy studies, a high protection against severe VCD cases (as assessed by IDMC) and hospitalized VCD cases was observed after at least 1 injection in subjects 9-16YOA in each individual study and in the pooled analysis of both studies (VE of 80.8% [95%CI: 70.1; 87.7] and 93.2 [77.3; 98.0] against hospitalized and severe VCD respectively).

Data on incidence of hospitalized and severe VCD were also collected from long-term follow up of the pivotal studies as a safety endpoint, showing during the first 3 years of Hospital Phase/SEP in CYD14 and CYD15 and from the entire Hospital Phase of CYD57 a continued reduction in the risk of hospitalized VCD and severe VCD in vaccinees 9-16YOA compared to placebo. Although a trend toward a higher relative risk was observed in the Hospital Phase compared to the Active Phase, the RR remains below 1 for the entire study period. The RR over a 5-year period in the pooled analysis was 0.361 (95%CI: 0.28; 0.46) against hospitalized VCD and 0.357 (95%CI: 0.20; 0.61) against severe VCD in subjects 9-16YOA.

At an individual level, the subjects' age at vaccination, previous exposure to dengue (dengue serostatus at baseline), and level of the response to the vaccine all had an effect on efficacy outcomes. Within these factors impacting VE, previous exposure to dengue was a key factor. Vaccine performance by dengue serostatus at baseline was estimated from the immunogenicity subset and NS1 supplemental study based on dengue anti-NS1 ELISA and PRNT50 assays. Vaccine efficacy against symptomatic VCD due to any serotype over the Active Phase (month 0 to month 25) was demonstrated in individual studies and in the pooled analysis in subjects with prior dengue exposure (dengue seropositive) at the time of vaccination. In the pooled analysis of the immunogenicity subset, VE against symptomatic VCD over 2 years after dose 1 was 81.9% (95%CI: 67.2; 90.0) in seropositive subjects 9-16YOA, which was consistent with findings from the NS1 supplemental study. Data from the NS1 supplemental study showed long-term (month 0 to month 60-72) protection against hospitalized dengue and severe dengue. In the analysis of pooled studies, all the estimated HRs/RRs were <1 and statistically significant.

Immune responses to the vaccine were evaluated in a subset of the population from the pivotal trials, showing robust humoral responses to 3 doses especially in seropositive 9-16YOA subjects at baseline. In adults 18-45YOA, post-injection 3 Ab levels were comparable to those seen in CYD14 and CYD15 where efficacy was demonstrated, indicating that protection is expected in adults living in endemics areas. A decrease in the GMTs against all 4 serotypes was observed one year after the third injection. Then, GMTs stabilize over the next 2 to 4 years and remain superior to pre-vaccination GMTs. The GMTs levels depend on age (increase with increasing age) and dengue serostatus at baseline (lower in seronegative subjects).

3.3. Uncertainties and limitations about favourable effects

In subjects 9-16YOA without prior dengue exposure (dengue seronegative at baseline), the meta-analysis results for vaccine efficacy against symptomatic VCD over the first 25 months from the immunogenicity subset of CYD14 and CYD15 showed a VE estimate at 52.5% (95% CI: 5.9; 76.1), which was broadly consistent with findings from the NS1 supplemental study based on anti-NS1 ELISA and PRNT50. This indicates some benefit in subjects without prior dengue exposure.

The vaccine is much less immunogenic in subjects dengue seronegative at baseline as compared to those seropositive, in terms of both GMT titres and percentage of subjects that seroconverted to all four dengue serotypes. Generally, a trend toward lower post dose 3 (PD3) GMTs was observed against serotype 1 compared to the 3 other serotypes, however PD3 GMTs varied widely across studies depending on serotype, region, age group, and baseline dengue immune status. The clinical relevance of these findings is unknown.

Vaccine efficacy against dengue varied across the four dengue serotypes, with lower efficacy for serotypes 1 and 2 than against serotypes 3 and 4 in the overall population regardless of baseline serostatus.

For the age group 17 to 45 years of age there are no clinical efficacy data, and immunogenicity data are limited. However post-dose 3 GMT can be used for immunobridging based on the evidence that robust immune response are generated following vaccination across age groups and trials, but the actual magnitude of efficacy relative to that observed in children and adolescents is unknown. Additional data will be collected in adults post-authorisation through effectiveness studies.

It is not currently feasible to distinguish baseline seropositivity to 2, 3 or 4 serotypes and thus it is not possible to investigate the effect that this may have on vaccine efficacy, especially in adults. Therefore it is important that data on adults is collected from post licensure effectiveness studies in endemic countries, based on vaccination implementation by countries in this age range.

There is no correlate of protection for dengue currently established however higher titres post-injection 3 of neutralising antibodies were found associated with a lower risk of dengue disease and higher vaccine efficacy.

The decision to select a three-dose vaccination schedule was based mainly on data in seronegatives in order to overcome poor immunogenicity in the seronegatives. Data on immunogenicity and efficacy after each dose in seropositive is limited and exploratory (due to high compliance) to allow to conclude on protective efficacy of seropositive individuals with less than three doses. Post-authorisation studies such as CYD65 may provide more information on immunogenicity and safety of one-dose and two-dose vaccination schedule.

There is limited data in individuals residing in non-endemic countries and no data in individual residing in non-endemic countries and traveling to endemic countries, therefore Dengvaxia should not be used for travellers even if seropositive. In addition Dengvaxia should not be used in the context of dengue outbreak in non-endemic regions.

Long term protection afforded by the vaccine is not known at this time. With the ongoing protocol amendment 4 of CYD14 and CYD15 studies (implementation of the Surveillance Expansion Phase [SEP] for the detection of VCD cases hospitalized or not), further prospective data on the full range of dengue disease both as safety and efficacy endpoints are being generated up to 5 years post-completion of the vaccine schedule. In addition, long-term protection from dengue will also be evaluated through Post Approval Effectiveness studies with a follow-up of 5 years as described in the RMP.

The need for additional booster doses remains to be elucidated. Three studies planned or ongoing (Studies CYD65, CYD63, CYD64) will investigate a booster dose at 1, 2, 4 or 5 years after the last injection.

There are no efficacy data in the age range of the indication on co-administration with other vaccines, hence coadministration is not recommended. Some data will be generated post-authorisation on coadministration with a booster dose of Tdap and HPV vaccines.

3.4. Unfavourable effects

In subjects 9 to 45 years of age, the most frequently reported reactions whatever the dengue serostatus prior to vaccination, were headache (54%), injection site pain (49%), malaise (44%), myalgia (43%), asthenia (34%), and fever (16%). In the paediatric population 9-17 YOA, fever has been observed with a higher frequency (very common) than in adults (common). Urticaria (uncommon) was only reported in subjects 9 to 17 years of age (none in adults).

Adverse reactions occurred within 3 days following vaccination except fever which appears within 14 days after the injection. The adverse reactions were usually mild to moderate in severity and of short duration (0 to 3 days).

Systemic adverse reactions tended to be less frequent after the second and third injections of Dengvaxia as compared to the first injection. Allergic including anaphylactic reactions have been reported very rarely.

Overall, the same adverse reactions but at lower frequencies were observed in dengue seropositive subjects.

3.5. Uncertainties and limitations about unfavourable effects

The analyses from the first year of Hospital Phase in CYD14 showed a higher incidence of hospitalized – and to a lesser extent severe- VCD cases in subjects 2 to 8 year-old in vaccinees compared to placebo, driven by subjects below 6 years of age at enrolment: RR of 7.454 in subjects 2 to 5 years (outside of the proposed indication). In CYD57, an excess of hospitalized VCD cases was also observed in subjects below 6 years of age, with a RR of 2.443 in subjects 4 to 5 years. Based on the NS1 Supplemental analysis that was used to infer baseline serostatus by anti-NS1 ELISA and PRNT50 assays for all hospitalized VCD in the pivotal trials, a trend towards an increased risk of hospitalized and severe dengue was identified in seronegative individuals only, including those aged 9-16 years over the entire study duration (RR 1.412 [95% CI: 0.743, 2.682] and RR 2.435 [95% CI: 0.472, 12.559] for hospitalised and severe dengue respectively in pooled studies). The increased risk against hospitalized VCD was mainly observed against serotype 1 and 2 in this age group and mainly during the 3rd year following the first injection. The clinical profile was similar between severe cases in the seronegative vaccine group and the seropositive placebo group. Based on these results, risk of hospitalized and severe dengue in individuals without prior dengue infection has been classified as an important identified risk in RMP and listed in the product information with a warning. The biological mechanism underlying such increased risk of severe dengue and hospitalization in seronegative subjects is unknown. Therefore adequate consideration should be given to the laboratory methods and tests that will be used to confirm prior dengue exposure before vaccination, in order to minimise the risk in the indicated population.

A risk of severe dengue disease due to waning of vaccine-protection over time is considered an important potential risk in the RMP and it will be followed up in post-marketing.

There are limited safety data in adults. In immunological and safety terms, subjects from 9 to 45 years of age are comparable to subjects 9-16 years of age, and therefore since no excess risk is observed for seropositive subjects 9 to 16 years old it is reasonable to assume that a similar situation would apply to 16 to 45 years-old. Additional safety data will be collected post-authorisation in adults through a long term safety study as per RMP.

No data are available in immunosuppressed subjects. The use in this population is contraindicated. However some data will be generated post-authorisation.

There are limited data from inadvertent use of Dengvaxia in pregnant women during clinical trials. Pregnancy was an exclusion criterion. These data are not sufficient to conclude on the absence of potential effects of Dengvaxia on pregnancy, embryo-foetal development, parturition and post-natal development, given that Dengvaxia is a live attenuated vaccine. There are no data available on breastfeeding. Therefore Dengvaxia is contraindicated during pregnancy and breastfeeding. A pregnancy registry will collect data post-authorisation.

There are very limited safety data regarding dengue vaccine interactions when administered sequentially or co-administered with other vaccines, therefore co-administration with other vaccines is not recommended at this stage. Some data will be generated post-authorisation.

Because Dengvaxia is based on yellow fever vaccine construct, there might be a potential risk of yellow fever vaccine-associated viscerotropic and neurotropic disease, which will be followed up via routine safety monitoring in the post-authorisation phase as well as in a cohort event monitoring study.

3.6. Effects Table

Table 39: Effects Table for Dengvaxia to prevent dengue disease in individuals 9 to 45 years of age with documented prior dengue virus infection and living in endemic areas.

Effect	Short description	Unit	Dengvaxia	Placebo	Strength of evidence/ Uncertainties	References
Favourable Effects						
Efficacy in 9-16 YOA (all)	VE against symptomatic VCD during PD3 period due to any of the 4 serotypes	VE % (95%CI) Cases/ person-years	62.8 (55.7; 68.8) 219/15657	-- 291/7742	N=24,858 primary objective met, any serostatus at baseline	Pooled CYD14+ CYD15
Efficacy in 9-16 YOA (sero+)	VE against symptomatic VCD during PD3 period due to any of the 4 serotypes	VE % (95%CI) Cases/ person-years	79.4 (58.4; 89.8) 11/1473	-- 26/713	N= 4,000 (immunogenicity subset) VE in baseline seropositive sbj	Pooled CYD14+ CYD15
Efficacy in 9-16 YOA (all)	VE against symptomatic VCD during Active Phase due to any of the 4 serotypes	VE % (95%CI)	65.6 (60.7; 69.9)	--	N=25,826 secondary objectives met VE was moderate for serotypes 1 and 2 and higher for serotypes 3 and 4	Pooled CYD14+ CYD15
Efficacy in 9-16 YOA (sero+)	VE against symptomatic VCD during Active Phase due to any of the 4 serotypes	VE % (95%CI)	81.9% (67.2 ; 90.0)	--	N= 4,000 (immunogenicity subset) VE in baseline seropositive sbj	Pooled CYD14 + CYD15
Efficacy in 9-16 YOA (all)	VE during Active Phase due to any of the 4 serotypes against: i) hospitalised VCD ii) severe VCD iii) DHF (WHO 1997)	VE % (95%CI)	i) 80.8 (70.1; 87.7) ii) 93.2 (77.3; 98.0) iii) 92.9 (76.1; 97.9)	--	N=25,826 secondary objectives met	Pooled CYD14 + CYD15
Efficacy in 9-16 YOA (sero+)	VE during Active Phase due to any of the 4 serotypes against: i) hospitalised VCD ii) severe VCD	VE % (95%CI)	i) 89.2 (78.5; 94.6) ii) 95.3 (68.9; 99.3)	--	exploratory analysis	Pooled CYD14 + CYD15+ CYD23
Unfavourable Effects^						
Systemi	Headache	Frequency of	54%	52%	Same ADRs at	Pooled*

Effect	Short description	Unit	Dengvaxia	Placebo	Strength of evidence/ Uncertainties	References
c ADRs 9-45YOA (all)	Malaise Myalgia Asthenia fever	reporting subjects in clinical trials (%)	44% 43% 34% 16%	37% 38% 31% 16%	lower frequencies were observed in dengue seropositive subjects; Frequency of Grade 3 ADRs: local 0.8%, systemic 9.0%	CYD14+ CYD15
Local ADRs 9-45YOA (all)	Injections site pain	Frequency of reporting subjects in clinical trials (%)	49%	39%		

Abbreviations:
Cases: number of subjects with at least one symptomatic virologically-confirmed dengue episode in the considered period.
Person-years: sum of time-at-risk (in years) for the subjects during the study period.
CI: confidence interval; VE: Vaccine Efficacy; PD3 period: the 12-month period starting from 28 days after the third injection; sero+: seropositive subjects at baseline; all: all study subjects regardless of serostatus at baseline
ADRs: adverse drug reactions
Notes: VE is calculated using density incidence (cases per 100 person-years at risk). The Active Phase represents the 25-month period after the first injection. * Reactogenicity subset of 1306 adults and 3067 children (9-45YOA). ^ Only the most frequently reported solicited local and general ADRs are reflected in the table. Refer to the SmPC for a full safety profile.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Dengvaxia showed protection against dengue disease in a large number of children aged 9-16 years, including protection against the severe forms of the disease which are considered the main purpose of a dengue vaccine. An extensive safety database indicates a favourable reactogenicity profile similar to other licensed vaccines.

Exposure to dengue virus before vaccination was identified as a key factor influencing vaccines efficacy. While exploratory data indicated that a potential limited short-term benefit against symptomatic VCD might be conferred by vaccination in individuals 9-16 years of age without prior dengue exposure, this benefit is offset by an apparent increased risk of hospitalized and severe dengue in the long term follow up period of the pivotal studies. Estimates from the long-term analysis suggest the onset of increased risk was mainly during the 3rd year following the first injection.

In 9 to 16 years old (and even in the 2 to 8 years old subjects, outside of the proposed indication) classified as seropositive at baseline, a decreased risk of hospitalized VCD and clinically severe VCD case was observed during the entire study. This decreased risk in seropositive subjects 9 to 16 years of age translated into an estimate of 15 hospitalized dengue cases or 4 severe dengue cases potentially prevented per 1000 vaccinees with previous dengue infection during 5 years of follow up from the first injection (based on cumulative incidence, seroprevalence and epidemiological conditions of the trials).

Although these figures may not be extrapolated to other regions with different seroprevalence and epidemiological situations, the vaccine would clearly provide a benefit to individuals with prior infection by dengue virus and the potential to prevent subsequent secondary severe infections.

During the procedure a Scientific Advisory Group (SAG) was convened to discuss the available evidence. The SAG concluded that even in areas where dengue is endemic, an age cut-off alone is not considered sufficient to minimise the risk of severe dengue seen in seronegatives and that, within the age range proposed by the Applicant for the indication (9-45 YOA), the vaccine should only be used in subjects who have a laboratory confirmed exposure to dengue virus. When deciding this, the SAG took into account the

fact that endemicity and seroprevalence are highly variable and changeable over time for dengue virus, and that even in areas affected by outbreaks both remain moderate in EU territories.

The SAG discussed that there are at least 3 key factors to consider when defining a population that may benefit from Dengvaxia: serostatus at baseline, endemicity and age. It was agreed that limiting the use of the vaccine to seropositive subjects 9-45YOA residing in endemic areas would be the best possible strategy under the current knowledge to limit the risk of developing severe dengue as seen in seronegative subjects. In light of the well-known heterogeneity of dengue epidemiology, a seroprevalence-driven vaccination policy is deemed impractical and not sufficiently reliable regardless of endemicity.

3.7.2. Balance of benefits and risks

In conclusion, this application presented data in children and adults from the age of 9 months to 60 years of age, with a proposed indication in the 9-45 years range. The overall available data demonstrate that there is benefit in preventing symptomatic and severe dengue disease by vaccinating individuals 9-45 years of age and that the reactogenicity profile is favourable.

However due to an increased risk of hospitalisation for dengue and clinically severe dengue (predominantly grade 1 or 2 Dengue Haemorrhagic Fever) that was identified in vaccinated seronegative subjects, the use of Dengvaxia should be restricted to individuals who experienced a prior dengue virus infection and who are living in endemic areas. Previous dengue infection has to be assessed before vaccination by laboratory confirmed history of dengue or through an appropriately validated serological test.

To avoid vaccination of false positives, only test methods with adequate performance in terms of specificity and cross-reactivity to other flaviviruses based on the local disease epidemiology should be used.

The RMP includes additional risk minimization activities to limit and prevent the identified risk.

3.8. Conclusions

The overall B/R of Dengvaxia is positive in individuals aged 9-45 years with laboratory confirmed prior dengue infection and living in endemic areas.

Divergent positions are appended to this report.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the benefit-risk balance of Dengvaxia is favourable in the following indication:

Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 to 45 years of age with prior dengue virus infection and living in endemic areas (see sections 4.2, 4.4 and 4.8). The use of Dengvaxia should be in accordance with official recommendations.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Dengvaxia in each Member State the Marketing Authorisation Holder (MAH) must agree the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each Member State where Dengvaxia is marketed, all healthcare professionals who are expected to use Dengvaxia have access to/are provided with the following educational package:

- Physician educational material

The physician educational material should contain:

- The Summary of Product Characteristics
- Guide for healthcare professionals

The Guide for healthcare professionals shall contain the following key elements:

- That there is an increased risk of severe and/or hospitalized dengue following vaccination in individuals not previously infected by dengue virus;
- That healthcare professionals have to document before vaccination the previous dengue infection, which has to be assessed by laboratory confirmed history of dengue or through serotesting;
- The healthcare professionals should be aware that the test they use should have adequate performance in terms of specificity and cross-reactivity based on the local disease epidemiology.
- That healthcare professionals should be aware of dengue early warning signs.

Divergent position(s) to the majority recommendation is appended to this report.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that chimeric yellow fever dengue virus serotypes 1, 2, 3 and 4 (live, attenuated) is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0174/2015 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

APPENDIX 1

DIVERGENT POSITION DATED 18 OCTOBER 2018

Divergent position Dengvaxia (EMEA/H/C/004171) – Dated 18 October 2018

The below mentioned members of the CHMP consider that: (i) the ability of serotests (currently on the market or in development) to exclude the vaccination of dengue-seronegative individuals is not enough known (ii) the committed risk minimization measures are insufficient. This leads to an undetermined risk of inadvertent vaccination of seronegative subjects, which precludes to conclude on a positive Benefit-Risk balance for Dengvaxia.

The rationale is as follows:

Use of the vaccine in subjects who did not yet acquire a natural immunity against dengue is associated with an identified risk for severe dengue, which can be potentially fatal. On the other hand, use of the vaccine in subjects previously exposed is associated with protection against dengue. Thus, it is key to univocally identify the dengue infection history of candidate vaccinees based on their medical records and/or their serostatus prior to vaccination.

The indication is now restricted to individuals with documented prior dengue virus infection. General recommendations on the importance of adequate testing are reflected in the indication and warning section of the PI. In addition the Applicant will prepare a guide for HCP in order to limit misuse in the field. However, despite these risk minimisation measures, the risk that a seronegative individual will ultimately be vaccinated and put at risk of potentially fatal dengue might not be contained. The below mentioned members of the CHMP therefore remain very cautious with regard to the potential public health implications of this dengue vaccine.

Only a test (testing strategy) leading to a negligible number of false positives (hence inadvertent vaccination of naïve individuals) should be used pre-vaccination. To achieve high Positive Predictive Values (PPV), a test with a specificity approaching 100% is required whatever the epidemiological context, because of inevitable uncertainties on seroprevalence levels, hence on pre-test probabilities (highly locally variable, variable over time, variable according to demographic characteristics).

The performances of CE certified anti-dengue IgG ELISA and Rapid Diagnostic Tests (RDT) tests that are commercially available are likely to be largely overestimated in various epidemiological contexts (flavivirus co-circulation and vaccination). In particular Zika cross-reactivity is not well characterized. At present, neutralisation assays are the most specific, but may also be affected by cross-neutralisation in the context of Zika co-circulation. A strategy combining multiple tests could improve the specificity for determining the dengue serostatus. However, up to which level of specificity (while maintaining acceptable sensitivity) the multiple testing strategy can lead remains unclear. Highly specific serotests are being developed through the use of new technologies (such as very specific epitopes) but it is unclear when such tests will be available.

Consequently, it is not clear whether and when tests (or testing strategies) with adequate performances would be both available and feasible within the European context. The below mentioned members of the CHMP consider that this constitutes an obstacle for granting MA. More specifically: (i) At present, the risk of inadvertently vaccinating naïve individuals is not known. This leads to large uncertainties on the actual benefit/risk balance, (ii) To achieve a negligible number of inadvertent vaccination of naïve individuals, a test with a specificity approaching 100% is required. It is even more uncertain if and when such test (testing strategy) is/would become available.

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