Australian Government
Department of Health
Therapeutic Goods Administration

Australian Public Assessment Report for live, attenuated, chimeric yellow fever dengue virus (serotypes 1, 2, 3 and 4)

Proprietary Product Name: Dengvaxia

Sponsor: Sanofi-Aventis Australia Pty Ltd

November 2018

TGA Health Safety Regulation

About the Therapeutic Goods Administration (TGA)

- The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health and is responsible for regulating medicines and medical devices.
- The TGA administers the *Therapeutic Goods Act 1989* (the Act), applying a risk management approach designed to ensure therapeutic goods supplied in Australia meet acceptable standards of quality, safety and efficacy (performance) when necessary.
- The work of the TGA is based on applying scientific and clinical expertise to decision-making, to ensure that the benefits to consumers outweigh any risks associated with the use of medicines and medical devices.
- The TGA relies on the public, healthcare professionals and industry to report problems with medicines or medical devices. TGA investigates reports received by it to determine any necessary regulatory action.
- To report a problem with a medicine or medical device, please see the information on the TGA website <https://www.tga.gov.au>.

About AusPARs

- An Australian Public Assessment Report (AusPAR) provides information about the evaluation of a prescription medicine and the considerations that led the TGA to approve or not approve a prescription medicine submission.
- AusPARs are prepared and published by the TGA.
- An AusPAR is prepared for submissions that relate to new chemical entities, generic medicines, major variations and extensions of indications.
- An AusPAR is a static document; it provides information that relates to a submission at a particular point in time.
- A new AusPAR will be developed to reflect changes to indications and/or major variations to a prescription medicine subject to evaluation by the TGA.

Contents

Common abbreviations

Abbreviation	Meaning
1/dil	Reciprocal of dilution
Ab	Antibody
ACV	Advisory Committee on Vaccines
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AND	Acute neurotropic disease
AP	Asia 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
DF	Dengue fever
DHF	Dengue haemorrhagic 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

Abbreviation	Meaning
FASI	Full analysis set for immunogenicity
FDA	Food and Drug Administration
FV	Flavivirus
GMT	Geometric mean titre
GMTR	Geometric mean of titre ratio
HSA	Human Serum Albumin
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
Latin America	Latin America
LMP	Last menstrual period
MedDRA	Medical Dictionary for Regulatory Activities
mFASE	Modified full analysis set for efficacy
MMR	Measles/mumps/rubella
MN	Microneutralisation
NS1	Non-structural 1
PoC	Proof of concept
PPSE	Per-protocol analysis set for efficacy
prM	Pre-membrane
PRNT	Plaque reduction neutralisation test
PT	Preferred term
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

Abbreviation	Meaning
SVCD	Severe virologically-confirmed dengue
VCD	Virologically-confirmed dengue
VE	Vaccine efficacy
WBC	White blood cells
WHO	World Health Organization
YF	Yellow fever

I. Introduction to product submission

Type of submission:	New biological entity (vaccine)
Decision:	Approved
Date of decision:	13 July 2017
Date of entry onto ARTG:	20 July 2017
ARTG number:	275964
Active ingredient:	Live, attenuated, chimeric yellow fever dengue virus (serotypes 1, 2, 3 and 4)
Product name:	Dengvaxia
Sponsor's name and address:	Sanofi-Aventis Australia Pty Ltd Talavera Corporate Centre Building D, 12-24 Talavera Road Macquarie Park, NSW 2113
Dose form:	Powder for suspension
Strength:	4.5 to 6.0 $\log_{10}$ $CCID_{50}$ for each serotype
Pack sizes:	Vaccine vial and diluent syringe with separate needles; pack sizes of 1 and 10
Approved therapeutic use:	*Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age with previous dengue infection and living in endemic areas.* *Use should be in accordance with official guidelines. Previous dengue infection must be demonstrated by history of laboratory-confirmed dengue infection or serotesting according to local official recommendations.*[1]
Route of administration:	Subcutaneous (SC) injection
Dosage:	The primary vaccination schedule consists of 3 injections of 0.5 mL to be administered by SC injection at 6 month intervals.

[1] Subsequent to approval submission PM-2016-01679-1-2, the indication was revised as a result of a Safety Related Request (SRR) by the sponsor (Submission PM-2017-04923-1-2) and a request for changes to the product information (PM-2017-04924-1-2). The indications approved with the initial submission were:

> *Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age living in endemic areas. Use should be in accordance with official guidelines (see 'Dosage and Administration').*

Please see the "Post outcome" section of this AusPAR for further details.

Product background

This AusPAR describes an application by the sponsor to register Dengvaxia as a new biological entity. This is a vaccine with a proposed indication 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.

Dengue disease is a mosquito-borne viral disease caused by four dengue virus serotypes closely related but antigenically distinct (serotype 1, 2, 3, and 4) transmitted primarily by the *Aedes aegypti* mosquito.[2] The infection often remains silent or can cause flu like illness that can evolve into a potentially lethal complication called severe dengue (including dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS)). 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.

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.[3] It is a major international public health concern, with nearly half of the world's population, in over 100 countries, at risk. It is a health priority in many countries where dengue disease is endemic and there is no specific treatment available. The only currently available prevention of dengue by vector control has proven to be of limited success, very difficult to sustain and costly.[4] Vaccination provides a viable and practical alternative in disease control measures. There is no specific treatment of Dengue infection; it is just supportive.

The CYD dengue vaccine is a sterile and freeze-dried product to be reconstituted before injection with sterile solution of 0.4% sodium. The vaccine is presented in a single-dose vial. The diluent is provided as a pre-filled syringe for single-dose presentation.

Before reconstitution, the vaccine is a white, homogenous, freeze dried powder with possible retraction at the base, and may form a ring shaped cake. The diluent is a clear, colourless liquid. After reconstitution, Dengvaxia is a clear, colourless liquid with the possible presence of white to translucent particles.

After reconstitution, each 0.5 mL dose contains approximately 5 $\log_{10}$ cell-culture (4.5 to 6) infectious dose 50% ($CCID_{50}$) per dose of each live, attenuated, dengue virus serotype 1, 2, 3 and 4.

The primary vaccination schedule consists of 3 injections of 1 reconstituted dose (0.5 mL) at 6 month intervals. The need for a booster dose after primary vaccination has not yet been established.

Once the freeze-dried vaccine has been completely reconstituted with the supplied diluent, it is administered via the subcutaneous (SC) route.

The recommended injection site is the deltoid region. Other injection sites may be recommended according to national guidelines.

Regulatory status

At the time of this submission, Marketing Authorisation Applications for Dengvaxia had been submitted in several dengue endemic countries from January 2015. The priority for

[2] Gubler DJ. Dengue. In: Epidemiology of arthropod-borne viral disease. Monath TPM, editor, Boca Raton (FL): CRC Press, 1988: 223-60.
[3] Kyle JL, Harris E. Global spread and persistence of dengue. *Ann Rev Microbiol.* 62: 71-92 (2008).
[4] Gibbons RV. Dengue conundrums. *Int J Antimicro Ag.* 2010: S36-S39; World Health Organization. Report of the 8th Meeting of the Global Collaboration on Development of Pesticides for Public Health (GCDPP), held in WHO/HQ, Geneva 20-21 February 2012.

submission was given to the countries with the highest disease burden. At time of submission, Dengvaxia had been approved in a total of 16 dengue endemic countries including Mexico, The Philippines,[5] Brazil, El Salvador, Costa Rica, Guatemala, Indonesia, Peru, Bolivia, Singapore, Cambodia, Thailand, Paraguay, Venezuela, Argentina and Malaysia. It has not been rejected or withdrawn in other countries. An application for Dengvaxia containing the same data set as this application was submitted to the EU.

Product Information

The Product Information (PI) approved with the submission which is described in this AusPAR can be found as Attachment 1. For the most recent PI, please refer to the TGA website at <https://www.tga.gov.au/product-information-pi>.

II. Registration timeline

The following table captures the key steps and dates for this application and which are detailed and discussed in this AusPAR and Attachment 2.

Table 1: Registration timeline

Description	Date
Submission dossier accepted and first round evaluation commenced	30 June 2016
First round evaluation completed	23 December 2016
Sponsor provides responses on questions raised in first round evaluation	31 January 2017
Second round evaluation completed	10 March 2017
Delegate's Overall benefit-risk assessment and request for Advisory Committee advice	1 May 2017
Sponsor's pre-Advisory Committee response	16 May 2017
Advisory Committee meeting	31 May 2017
Registration decision (Outcome)	13 July 2017
Completion of administrative activities and registration on ARTG	20 July 2017
Number of working days from submission dossier acceptance to registration decision*	236

* Legislative timeframe for standard applications is 255 working days.

[5] After being granted market authorisation in The Philippines on 22 December 2015, the licence was subsequently suspended until 2 January 2019.

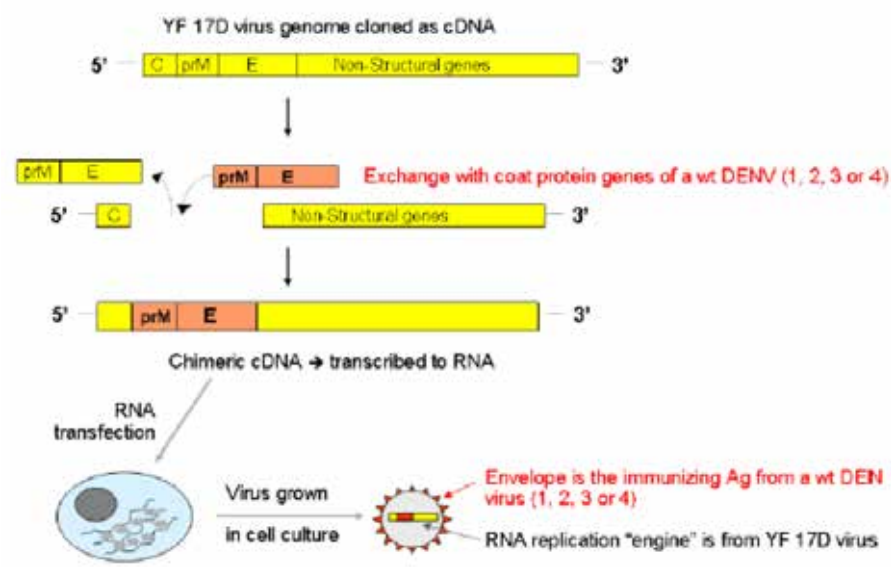
III. Quality findings

Drug substance

CYD dengue vaccine is a tetravalent, live attenuated viral vaccine. 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.

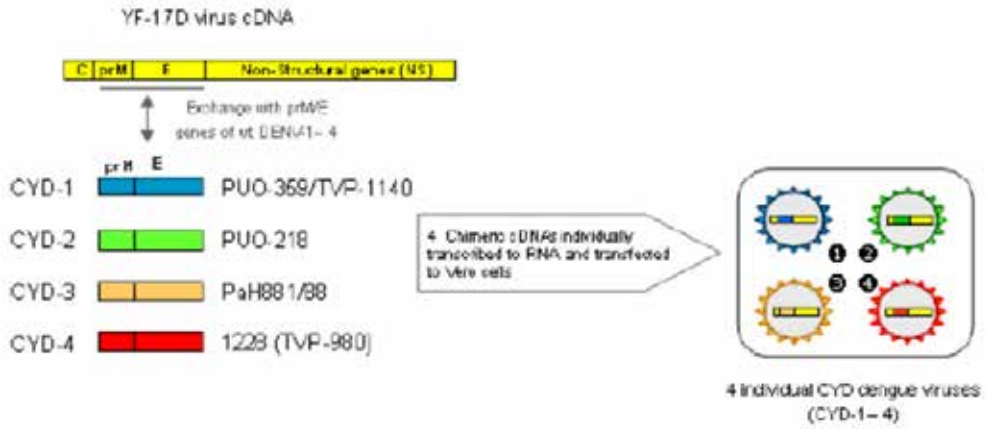
As shown below, CYD dengue viruses were constructed by replacing the sequence encoding the prM and E structural ('coat') proteins in YF-17D virus genome by those encoding for the homologous sequences of the four wt dengue serotypes 1 (PUO-359/TVP-1140), 2 (PUO-218), 3 (PaH881/88), and 4 (1228/TVP-980). No additional sequences were added.

Figure 1: Construction of recombinant complementary deoxyribonucleic acid (cDNA) of each CYD dengue virus



This led to construction of four chimeric viruses (CYD 1-4, one for each serotype) as shown below, 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 will be determined mainly by the YF-17D virus replication engine. The immunising antigens are the prM and E proteins from the wt dengue viruses (serotype 1 to 4).

Figure 2: CYD dengue viruses 1-4 constructs



It is noted that 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.

Drug product

The following product attributes are critical for the performance of the vaccine:

- *Virus potency:* Main biological property of DP which is ensured by the measurement of CYD dengue virus concentration.
- *Sterility:* Sterility is important for injected vaccines, is ensured by validated aseptic process simulation and a validated sterilising filtration process.
- *Physicochemical properties of DP:* These properties are associated to excipients and diluent. The appearance of vaccine before reconstitution is a white homogenous freeze dried product. After reconstitution with the diluent, the pH value is within 7.1 and 8.1 and the osmolality value is between 400 mOsmol/kg and 800 mOsmol/kg.

The biological and physicochemical properties of the vaccine mentioned above are assessed by the release tests (refer to Drug Product (DP) specifications).

It is to be noted that 'Particles and filaments' at both DS and DP stages on CYD dengue samples were observed and were considered the result from the aggregation of endogenous proteins of intrinsic characteristics (that is, proteins of Vero cell origin). Presence of 1 particle/dose in Dengvaxia vaccine is considered as an intrinsic characteristic of CYD dengue vaccine based on:

- Number of these particles is limited to 1 per vial
- Residual DNA release limit is far below 10 ng/dose (complying with Ph. Eur.)
- Residual HCP are well characterised and the levels of protein content are very low in the final product
- Clinical studies as well as repeat dose toxicity studies did not show any cause of concern (TGA clinical evaluation and TGA nonclinical evaluation)
- The endogenous particles in commercial product are controlled through vaccine release specifications
- Quality control process performed at release of final freeze-dried product are able to distinguish the number of particle per vial and if the vaccine contains these endogenous particles or exogenous particles/filaments

- The description of the reconstituted vaccine included in the PI, is considered adequate for end user to distinguish these endogenous particles form the exogenous and other particles and filaments.

Overall, supplied data is satisfactory and there are no further quality related concerns pertaining to this issue. It should be noted that safety signals are beyond the quality evaluation report. For safety evaluation of this product, please refer to the clinical evaluation report.

Stability

Drug substance

The sponsor proposed a shelf life of 48 months at ≤ -70°C.

Stability data have been generated under real time and stressed conditions.

Stability data were generated under real time conditions to characterise the stability profile of the substance and to establish a shelf life. The real time data submitted support a shelf life of 48 months when stored at ≤ -70°C.

Drug product

Stability data have been generated under stressed and real time conditions to characterise the stability profile of the product. The freeze dried product should be stored protected from the light exposure once taken out its secondary packaging. Photostability data showed the packaged product is photostable.

The proposed shelf life is 36 months when stored at 2 to 8°C.

In-use stability data have also been submitted. The proposed shelf life and storage conditions for the reconstituted product are 6 hours when stored at 2 to 8°C.

The results remained acceptable at + 25°C ± 2°C only up to 3 months.

Data generated at + 37°C ± 2°C showed results were not acceptable at this temperature.

Stability studies have been conducted in accordance with relevant International Conference on Harmonisation (ICH) guidelines.

There are no issues pertaining to stability of DS or DP.

Quality summary and conclusions

There are no objections to the registration of this product from sterility; endotoxin, container safety and viral safety related aspects.

Overall, sufficient evidence has been provided to demonstrate that the risks related to the manufacturing quality of Dengvaxia Dengue tetravalent vaccine (live, attenuated) have been controlled to an acceptable level.

There are no further objections to the registration of Dengvaxia Dengue tetravalent vaccine (live, attenuated). However, it should be noted that the DP contains endogenous particles/filaments. There are no further concerns related to quality of these particles/filaments but safety signals of these particles/filaments are beyond this quality evaluation report and this issue requires the Delegate's consideration.

Proposed conditions of registration

It is a condition of registration that all independent batches of Dengvaxia Dengue tetravalent vaccine (live, attenuated) imported into Australia are not released for sale until samples and the manufacturer's release data have been assessed and you have received notification from the Laboratories Branch, TGA, that there is no objection to you releasing the product to the Australian market.

For each independent batch of the product imported into Australia, the sponsor must supply the following:

- A completed Request for Release Form.
- Complete summary protocols for manufacture and QC, including all steps in production.
- At least 5 doses of each first consignment of product lot with the Australian approved labels, PI and packaging. 3 doses of any further consignment of already released product (including diluents) with the Australian approved labels, PI and packaging.
- Certificate of Release from a regulatory agency acting for the country of origin such as an OMCL (if available).
- Any reagents, reference material and standards required to undertake testing, as requested by Laboratories Branch, TGA.

Distribution of each shipment of each batch of vaccine is conditional upon fulfilment of these conditions and receipt of a notification letter from the Laboratories Branch.

Samples and data should be forwarded to the Immunobiology Section, Laboratories Branch before release of each batch and with sufficient lead time to allow for Laboratories Branch testing.

Certified product details

An electronic copy of the Certified Product Details (CPD) should be provided upon registration of the therapeutic good. In addition, an updated CPD should be provided when any changes to finished product specifications and test methods are approved in an application or notified through a self-assessable change. The CPD templates are available on request and the completed form should be sent as a single bookmarked PDF document as soon as possible after registration/approval of the product or any subsequent changes as indicated above.

IV. Nonclinical findings

Introduction

The sponsor has applied to register a live attenuated tetravalent dengue (DEN) vaccine, Dengvaxia. Dengvaxia is indicated for the prevention of dengue disease caused by DENV serotypes 1, 2, 3 and 4 in individuals 9 through 60 years of age living in endemic areas. The primary vaccination schedule consists of 3 injections of 0.5 mL containing 4.5 to 6.0 $\log_{10}$ cell culture infectious dose ($CCID_{50}$) of each serotype (1-4), to be administered by SC injection at 6 month intervals. It is the first tetravalent dengue vaccine to be approved in some countries.

The WHO has issued general nonclinical guidelines for vaccines;[6] and guidelines specific to live attenuated dengue vaccines.[7] The EMA has issued a guideline for live recombinant viral vectored vaccines.[8] All nonclinical safety studies were Good Laboratory Practice (GLP) compliant. The studies were performed mainly in non-human primates (NHPs). Mice and rabbits were used for the reproductive toxicity studies.

Pharmacology

Dengue virus

Dengue virus is a member of the *Flavivirus* genus within the family *Flaviviridae,* which contains over 70 viruses, including yellow fever (YF), Japanese encephalitis, tick borne encephalitis, Zika, West Nile, Kunjin and Murray valley encephalitis. The amino acid homology between the 4 dengue serotypes is 63 to 68%, and 44 to 51% between dengue, yellow fever and West Nile viruses.[9]

DEN epidemiology in Australia

DEN has been reported in most Australian States and territories but locally acquired DEN and the mosquitoes that transmit DENV have only been confined to North Queensland. In Australia the first recorded outbreak to DEN occurred in 1879, the first fatality occurred in Charters Towers in 1885 and the first fatality attributed to severe dengue occurred in the same town during the 1897 epidemic, when 60 fatalities were recorded (30 of those were children). DEN is not endemic in Queensland but outbreaks all begin with a single imported case. Proportion of overseas acquired DEN in Queensland by serotype is as follows: 37% of DEN-1, 30% DEN-2, 22% of DEN-3 and 10% of DEN-4. The number of both imported and locally acquired cases has increased over the years as seen below.

Table 2: Dengue notifications by place of acquisition for QLD 2005 to 2014

Year	Locally acquired	Proportion locally acquired	Overseas acquired	Proportion overseas acquired	Not stated / unknown	Proportion not stated / unknown	Total
2005	76	66%	38	33%	2	1%	116
2006	37	49%	35	46%	4	5%	76
2007	47	39%	68	57%	4	3%	119
2008	127	55%	98	43%	4	2%	229
2009	915	89%	108	11%	3	< 1%	1026
2010	79	27%	206	72%	3	1%	288
2011	69	37%	117	63%	0	-	186

[6] WHO (2005). Guidelines on nonclinical evaluation of vaccines. WHO Technical Report Series, No. 927.
[7] WHO (2013). Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). WHO Expert Committee on Biological Standardization. WHO Technical Series No. 979, Annex 2.
[8] EMA CHMP (2010). Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (EMA/CHMP/VWP/ 141697/2009).
[9] Guirakhoo F, et al. Recombinant Chimeric Yellow Fever-Dengue Type 2 Virus Is Immunogenic and Protective in Nonhuman Primates. *J Virol.* 74: 5477-5485 (2000).

Year	Locally acquired	Proportion locally acquired	Overseas acquired	Proportion overseas acquired	Not stated / unknown	Proportion not stated / unknown	Total
2012	28	11%	214	88%	2	1%	244
2013	222	45%	267	54%	1	< 1%	490
2014	182	46%	213	54%	0	-	395
Total	1782	56%	1364	43%	23	1%	3169

The trend of dengue virus infection notifications (a mix of local and imported cases), received from throughout Australia (State and Territory health authorities) have generally increased steadily in frequency and intensity over the past 5 years as shown below.

Table 3: Dengue notifications by States and Territories 2012 to 2016

Year	ACT	NSW	NT	QLD	SA	TAS	VIC	WA	AUST
2012	22	290	68	244	51	8	330	527	1540
2013	12	300	56	487	75	19	412	480	1841
2014	16	377	62	393	72	17	334	450	1721
2015	19	344	55	264	74	19	385	554	1714
2016	34	388	88	354	108	31	447	522	1972

Dengue infection in humans

The majority of dengue infections are asymptomatic. Dengue fever (DF) is commonly characterised by sudden onset fever, severe headache, retro-orbital pain, generalised myalgia and arthralgia, abdominal pain and nausea, rash on the trunk and medial aspect of the arms and thighs, minor bleeding, leukopenia and thrombocytopenia, and hepatitis. Symptoms usually last for 2 to 7 days. In about 1 to 3% of cases the more severe forms may ensue:- dengue haemorrhagic fever (DHF), with marked thrombocytopenia; and dengue shock syndrome (DSS), with hypotension, plasma leakage, increased haematocrit, pleural effusion, bleeding, and organ impairment, which can be fatal.

Infection confers life-long immunity against infection with the same serotype, and transient protection against the other 3 serotypes for 2 to 3 months. Once cross-immunity from the primary infection wanes, a secondary dengue infection to a heterologous serotype may occur, with an approximate 7 fold increased risk of severe disease. Symptomatic disease with a third or 4th infection is rare. Severe disease has been associated with high levels of viraemia.[10]

Severe disease upon secondary heterologous dengue infection involves complex immunopathological responses which are not fully understood. Long standing hypotheses are antibody-dependent enhancement (ADE) of disease, in which non-neutralising antibodies facilitate virus uptake by cells via Fcγ receptors, and/or a misdirected inflammatory (cytokine) and/or T cell response. Severe disease may also occur with a primary infection in infants of dengue-immune mothers, suggesting the importance of

[10] Libraty DH, et al. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus activation. *J Infect Dis.* 185: 1213-1221 (2002).

antibodies in disease enhancement.[11] The phenomenon of 'original antigenic sin', in which antibody responses to a second infection are dominated by those specific to the virus serotype that caused the primary infection, has been reported for dengue;[12] it has also been reported for influenza.

There is no accepted immune correlate of dengue protection in humans. Dengue infection induces innate antiviral, antibody and cell-mediated responses. Neutralising antibody responses are directed primarily to the dengue E and PrM proteins, and higher levels of neutralising antibodies have been associated with protection from symptomatic infection in endemic areas.[13] Dengue infection also elicits serotype-specific T cell responses, major T cell epitopes are located on the dengue NS3 and NS1 proteins;[14] which have been replaced in CYD vaccine by the corresponding YF gene segments.

Animal models for DEN

There are currently no animal models that completely mimic the pathogenesis of DENV as seen in humans (that is, DEN fever syndrome ± haemorrhage, DEN haemorrhagic fever (plasma leakage) ± DEN shock syndrome). Humans, nonhuman primates, and mosquitoes are the only natural hosts of DENV. Nonhuman primates (such as macaques, monkeys, baboons and chimpanzees) are susceptible to DEN infection (and show detectable viraemia and antibody response), however they do not manifest clinically apparent or sub clinically detectable dengue diseases as seen in humans.[15] Therefore currently nonhuman primates are the only recommended species for evaluating replication, immunogenicity and neurovirulence of candidate DENV vaccines. The nonclinical vaccine studies used rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) monkeys, rhesus monkeys have been the most widely used in dengue vaccine research, some dengue vaccines have also been studied in cynomolgus monkeys.

An important limitation of the NHP models is that they do not show overt evidence of disease after SC dengue inoculation, which typically elicits levels of viraemia several orders of magnitude lower than in infected humans (approximately 1 to 3 $\log_{10}$ versus approximately 6 to 8 $\log_{10}$ PFU/mL). However, in rhesus macaques, abnormalities in blood biochemistry and coagulation have been reported after SC challenge with 10^5 PFU despite the absence of overt clinical symptoms;[16] and rash and haemorrhage has been reported after high IV challenge with 10^7 PFU, which elicited levels of viraemia 1 to 2 $\log_{10}$ units below that noted in patients with severe disease.[17] Rhesus and cynomolgus monkeys are susceptible to YF infection and disease, and are used to test neurovirulence of YF vaccines.

Several mouse dengue models have been described but none mimic clinical symptoms as seen in humans, wt viruses replicate with very low titres and therefore the use of mice as a true model for DENV in vaccine development is limited. However, mouse-brain adapted DENVs can induce fatal encephalitis after intracranial (IC) inoculation of suckling mice. Adaptation of a DENV-2 isolate to neurovirulence in suckling mice correlated positively

[11] Halstead SB. Dengue. *Lancet* 370: 1644-1652 (2007).
[12] Halstead SB, et al. Original antigenic sin in dengue. *Am J Trop Hyg.* 32: 154-156 (1983).
[13] Katzelnick LC, et al. Neutralising antibodies against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. *Proc Natl Acad Sci.* 113: 728-733 (2016).
[14] Mathew A, et al. Dominant recognition by human CD8+ cytotoxic T lymphocytes of dengue virus nonstructural proteins NS3 and NS1.2a. *J Clin. Invest.* 98: 1684-1691 (1996).
[15] Bente DA, Rici-Hesse R. Models for DENV infection. *Drug Discovery Today Dis Models* 3: 97-103 (2006).
[16] Hickey AC, et al. Serotype-specific host responses in rhesus macaques after primary dengue challenge. *Am. J. Trop. Med. and Hyg.* 89: 1043-1057 (2013).
[17] Onlamoon N, et al. Dengue virus-induced hemorrhage in a nonhuman primate model. *Blood* 115: 1823-1834 (2010).

with virulence in humans.[18] Suckling mice were used to assess the neurovirulence of CYD vaccines in order to demonstrate consistency during production.

Immunogenicity and viraemia

Immunogenicity: neutralising antibodies

The capacity of CYD vaccine to induce serum neutralising antibodies against the 4 dengue serotypes was investigated in NHP studies. Neutralising antibodies against homologous CYD dengue viruses and/or wt parental strains were measured in earlier studies by the plaque reduction neutralisation test (PRNT), using homologous (vaccine) virus. Titres were expressed as the highest serum dilution inhibiting 50% of the plaques ($PRNT_{50}$). Later studies used the seroneutralisation 50 (SN_{50}), a limit dilution assay based on the $CCID_{50}$ test, using Mahidol strains DEN-1 16007, DEN-2 16681, DEN-3 16562 and DEN-4 1036, these clinical isolates were not the parental strains. Assays were conducted in Vero cells. Data in the 2 assays were correlated ($R^2 \geq 0.92$). $PRNT_{50}$ values were 2- to 3-fold higher than SN_{50} values. All monkeys were tested for flavivirus seronegativity prior to vaccine treatment.

An early study (Study SBi-0946-88) in rhesus monkeys with monovalent CYD-1, 3 or 4 showed induction of neutralising antibodies to homologous virus in all monkeys 30 days after a single SC dose of approximately 5 $\log_{10}$ PFU. The tetravalent CYD vaccine induced neutralising antibodies to all 4 serotypes, a second dose on day 63 increased neutralising antibodies to all 4 serotypes in all monkeys by day 94, with no viraemia.

In cynomolgus monkeys, a single SC dose of approximately 5 $\log_{10}$ PFU of monovalent CYD-1, 2, 3 or 4 induced serum neutralising antibody titres in all monkeys by 30 days post-dose, titres to homologous virus were higher than to wild-type parent for all 4 serotypes. Antibody titres to homologous virus varied with serotype, with anti-DEN-1 titres approximately equal to DEN-2 > DEN-3 > DEN-4. Monovalent CYD also induced neutralising antibodies that were cross-reactive to heterologous serotypes (Study DEN020Mk). A single SC dose of approximately 10^5 PFU of the tetravalent vaccine induced neutralising antibody titres to all 4 serotypes in all monkeys by 30 days post-dose. Neutralising antibodies were induced in all monkeys despite viraemia being undetectable in some monkeys (Study SBi 131-88).

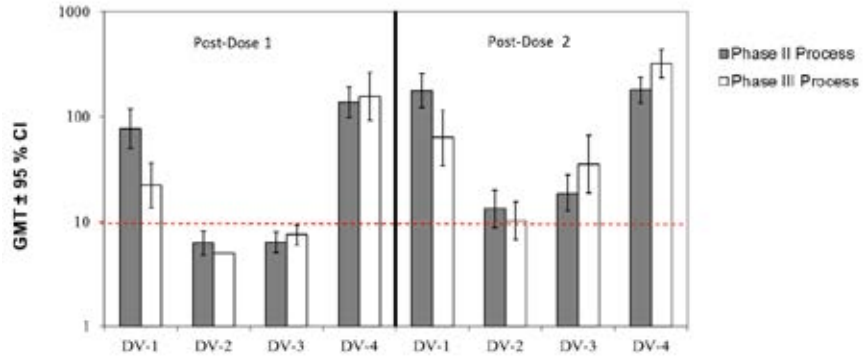
Single mixed SC doses of tetravalent CYD vaccine (5555, 3553, 5553, 3333 formulations, where 3333 = 10^3, 10^3, 10^3, 10^3 $TCID_{50}$ for CYD-1, 2, 3, and 4, respectively) induced seroconversion ($PRNT_{50}$>10) to all 4 homologous viruses in 6/6, 3/6, 4/6 and 6/6 cynomolgus monkeys, respectively, by day 31 post-dose, and in 6/6, 5/6, 4/6 and 6/6 monkeys, respectively, by day 121 post-dose. When vaccinated monkeys were challenged SC with wt DENV-1, 2, 3 or 4, all 24 monkeys showed marked increases in neutralising antibody titres by 30 days post-challenge, indicating an anamnestic (memory) response, and 22/24 were fully protected in terms of absence of viraemia (Study SBi 1324-88). A strong anamnestic antibody response 8 months after a booster dose was also seen after a high IV challenge dose in a second study (Study DEN020Mk/C3).

The effect of a second SC dose of ~5 $\log_{10}CCID_{50}$ of monovalent CYD-1, 2, 3 or 4 or tetravalent CYD two months after the first dose was tested in cynomolgus monkeys in Study DEN010Mk. Monovalent CYD vaccines induced neutralising antibody titres to the homologous serotypes, and no detectable viraemia, after one or two doses. Antibody responses to serotypes 1 and 4 were generally higher than to serotypes 2 and 3 with both the monovalent and tetravalent vaccines. Although some boosting occurred with the second dose, there were still some non-responders to serotypes 2 and 3. The

[18] Sabin AB, Schelesinger RW. Production of immunity to dengue with virus modified by propagation in mice. *Science* 101: 640-642 (1945).

predominance of serotypes 1 and 4 was observed in other studies, results of a meta-analysis of 8 studies with a total of 40 monkeys are shown in the figure below.

Figure 3: Antibody neutralisation titres (SN_{50}) induced by Phase II and III tetravalent CYD lots in NHPs

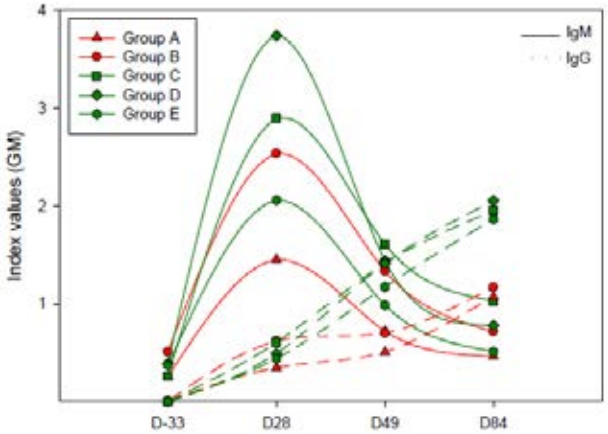


Interference between serotypes has been reported for other live attenuated dengue vaccines, and other live virus vaccines. Its causes were not fully elucidated, but involved differences between serotypes in replication, and intrinsic immunogenicity. CYD-1-4 interference prompted further studies with the objective of a more balanced immune response (below).

Duration of neutralising antibody response in NHPs

The dengue-specific IgG and IgM responses of cynomolgus monkeys to 2 consecutive monovalent CYD-2 or tetravalent CYD doses, 2 months apart, were measured by ELISA in Study DEN020Mk. Baseline IgM titres were variable, and elevated in some monkeys. After background value subtraction, IgM and IgG titres were shown to increase in all groups, but had different kinetics, with IgM titres rapidly peaking by day 28 and then declining, whereas IgG titres significantly increased from days 28 to 49 and 84 (last measurement), and were higher with the tetravalent vaccine (following figure).

Figure 4: Dengue-specific IgM and IgG responses after 1 or 2 CYD immunisations in cynomolgus monkeys.



(Groups A and B = monovalent CYD-2 vaccine, Groups C, D, E = tetravalent CYD vaccine)

The longest periods over which neutralising antibody titres were measured in cynomolgus monkeys were 8 months between the second vaccine and challenge doses in a challenge Study (DEN020Mk/C3), and 1 year between the second and third vaccine doses in an interference Study (DEN014Mk). There were no decreases in the numbers of seropositive

monkeys, and although not analysed quantitatively, there appeared to be no consistent declines in neutralising antibody titres, over these periods.

Immunogenicity; balancing serotypes

Three studies in cynomolgus monkeys (DEN011Mk, DEN012Mk and DEN014Mk) investigated different immunisation regimens to achieve a more balanced immune response against all 4 serotypes, in terms of antibody titres and/or seroconversion rate.[19] Sequential (2 months) complementary SC immunisation with bivalent CYD-1-2 followed by CYD-3-4, or CYD-1-4 followed by CYD-2-3, or simultaneous bivalent administration of CYD-1-2 and CYD-3-4 in separate arms, a 2 month interval between prime and boost, or a lower dose of CYD-4 (5553 versus 5555 formulation), all limited the dominance of serotypes 1 and 4 to some extent. Priming with YF 17D vaccine followed by tetravalent CYD vaccine also had a positive effect, although less pronounced than with 2 sequential bivalent doses. The positive effect of heterologous pre-immunity may have been a consequence of an anti-YF NS response, or cross reactivity against the dengue E epitopes (Study DEN011Mk). A tetravalent preparation of inactivated, purified virions (20 µg each, AF04 adjuvant) induced dominant responses for serotype 1, and to a lesser extent 3, suggesting that serotype 1 was intrinsically immunodominant, and the serotype 4 dominance in the live vaccine was due to higher replication (Study DEN016Mk).

A third dose one year after the first dose was the most successful regimen, although neutralising antibody titres of serotype 2 were still the lowest, followed by serotype 3, as shown in the following table (Study DEN014).

Table 4: Neutralising antibody titres (SN_{50}) in cynomolgus monkeys after 3rd dose at 1 year with tetravalent CYD

Group	Monkey ID	Initial Immunizations D0	Initial Immunizations D56	D371 (pre-1 year boost) DEN-1	DEN-2	DEN-3	DEN-4	1 year boost + 28 days DEN-1	DEN-2	DEN-3	DEN-4
1	AR465	TV 5/5/5/5	TV 5/5/5/5	252	63	13	319	3208	402	200	1010
	AR568			126	<	20	252	1010	50	126	637
	AR569			20	<	<	80	402	<	63	252
	AR639			126	<	16	126	1010	20	126	798
	GMT			95	<	12	169	1071	38	119	600

The need for booster doses to balance immunogenicity has been reported with some other live dengue vaccines.[20] The CYD vaccine data were the basis of the proposed 3 dose regimen in humans, however it failed to provide equivalent immunogenicity to all 4 serotypes in clinical trials.

Immunogenicity – consistency between phase I, II and III lots

The consistency of the immunogenicity of Phase I, II and III CYD vaccine lots in terms of neutralising antibodies and/or viraemia in NHPs was shown in studies DEN010Mk, DEN012Mk, DEN016Mk, DEN020Mk and DEN020Mk/C3.

Immunogenicity – YF backbone

No nonclinical data on immune responses to the vaccine YF17D vector backbone were submitted, although recommended by the EMA guideline.

[19] Guy B, et al. Evaluation of interferences between dengue vaccine serotypes in a monkey model. *Am J Trop Med Hyg.* 80: 302-311 (2009).
[20] Koraka P, et al. Efficacy of a live attenuated tetravalent candidate dengue vaccine in naïve and previously infected cynomolgus macaques. *Vaccine* 25: 5409-5416 (2007).

Immunogenicity - cell-mediated

No nonclinical data on cell-mediated immunity were submitted, although recommended by the EMA guideline. In a Section 31 response, the sponsor has stated that cell-mediated response studies were not carried out in monkeys but were done in clinical studies.

In seronegative humans, CYD vaccine was reported to induce serotype-specific T-helper and T-cytotoxic cell responses to all 4 serotypes after 3 doses (serotype 2 and 4 responses dominated after 1 injection), and a specific $CD8^+$ T cell response against YF17D non-structural NS3 antigen. Cross-reactivity against dengue NS3 was low.

Viraemia

Viraemia was measured in all the primary pharmacology studies in NHPs. In the earlier studies it was measured in plaque forming units (PFUs), which measure infectious virus, later studies utilised more sensitive qRT-PCR, 1 $\log_{10}$PFU was equivalent to about 3 $\log_{10}$ genome equivalent (GEQ/mL). The limit of quantification was 3 $\log_{10}$ GEQ/mL. Viraemia was measured over 10-14 days post-dose.

The viraemia dose-response after a single SC dose of 2, 3, 4 or 5 $\log_{10}$ PFU of monovalent CYD-2 was investigated in rhesus monkeys, there was no significant difference in the peak level of viraemia between dose levels, with mean peak titres ranging from 1.3-1.6 $\log_{10}$ PFU/mL, the mean duration of viraemia was 1 day longer in the HD group (4.25 ±1.7 days) than in the LD group.[21] The original study report for this study by Acambis was not available.

A single SC inoculation with monovalent CYD-1, 3, 4 or tetravalent CYD vaccine in rhesus monkeys resulted in in mean peak viraemia of 1.8-2.7 $\log_{10}$ PFU/mL (3.7 to 7 days duration) in the monovalent groups, and 2.8 $\log_{10}$ PFU/mL (2.8 days duration) in the tetravalent group. Mean peak viraemia elicited by monovalent or tetravalent CYD was similar to that with YF-VAX, and significantly lower than with a tetravalent preparation of wt DENV. Serotype 4 was most commonly detected after inoculation with tetravalent CYD. No viraemia was observed after a second dose of tetravalent CYD two months later (Study SBi 0946-88).

In cynomolgus monkeys, low levels of transient viraemia were observed in all studies with tetravalent CYD vaccine, typically between 3.5 to 5 $\log_{10}$ GEQ/mL, and not exceeding 7 days duration. Serotype 4 was predominant, as in rhesus monkeys. Results of a meta-analysis of viraemia in 8 studies with a total of 40 cynomolgus monkeys are shown below.

Table 5: Detection of CYD-1, 2, 3 or 4 viruses in cynomolgus monkeys vaccinated with Phase II or III lots.

Virus	CYD detection			Mk with viremia*	Detection peak		
	positive/tested*	*%*	*nb of days/mk*	*% (nb)*	*day*	*% mk*	*$\log_{10}$ GEQ/mL*
CYD1	6 / 36	17	1.4 ± 0.5	6 (2)	2	100	3.3 ± 0.3
CYD2	5 / 36	14	1.8 ± 1.0	6 (2)	2	80	3.3 ± 0.3
CYD3	10 / 36	28	1.4 ± 0.7	8 (3)	2	100	3.4 ± 0.2
CYD4	36 / 36	100	4.5 ± 1.6	97(35)	2 9	73 70	3.6 ± 0.5 3.7 ± 0.5

In pooled clinical studies, few vaccinated subjects (3.8%) had viraemia, the incidence of viraemia was lower with the second injection, and almost none was detected after the third. Viraemia generally occurred around day 7 and never after Day 14. CYD-4 was the

[21] Guirakhoo F, et al. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. *J Virol.* 74: 5477-5485 (2000).

most frequently identified vaccine serotype, as in monkeys. Viraemia was low regardless of the dengue immune status at baseline, and the age group.

Viraemia: CYD attenuation

Each of the CYD viruses is a chimera consisting of unmodified wt dengue E and prM structural sequences, which determine cell tropism, combined with the YF 17D non-structural sequences, which largely determine virus replication. The YF 17D backbone virus is attenuated from the wt Asibi parental strain by virtue of 22 amino acid substitutions.

Published data showed that monovalent CYD-1, 3 or 4 generally elicited a lower mean duration and peak titre of viraemia than the parent wt DENV in rhesus monkeys.[22] The original study report for this study by Acambis was not available.

Table 6: Viraemia in rhesus monkeys immunised SC with monovalent CYD or parent wt DENV.

Vaccine dose (log_{10}PFU)	Peak titre (log_{10}PFU/mL) (SD)	Duration (days)
YFD1 (4.3)	0.7 (0)	1.3 (0.6)
YFD3 (3.6)	1.3 (0.4)	1 (0)
YFD4 (3.8)	1.4 (1)	3 (2)
wt DENV-1 (PUO359) (3.9)	3.0 (0.9)	3.3 (2.1)
wt DENV-3 (PaH881/88) (5.2)	2.8 (0.5)	2.7 (1.1)
wt DENV-4 (1228) (3.8)	2.2 (0.15)	3.3 (0.6)

Tetravalent CYD elicited a significantly lower mean peak titre than tetravalent wt DENV, and a similar mean peak to YF-VAX in rhesus monkeys (Study SBi 0946-88) (see Table 7).

Table 7: Viraemia in rhesus monkeys immunised SC with tetravalent CYD, wt DENV or YF-VAX.

Vaccine dose, log_{10}PFU	Peak titre (log_{10}PFU/mL)	Duration (days)
Tetravalent CYD (CYD1,2,3,4 = 4.5, 3.0, 3.6, 4.4)	2.8	6
Tetravalent wt DENV (DENV1,2,3,4 = 4.4, 4.0, 5.4, 4.8)	4.3	8.5
YF-VAX (5.5)	2.3	3.7

Low levels of transient viraemia were also observed in all studies in cynomolgus monkeys, typically between 3.5to 5 log_{10} GEQ/mL, and not exceeding 7 days duration. Viraemia was

[22] Guirakhoo F, et al. Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. *J. Virol.* 75: 7290-7304 (2001).

within acceptable limits according to WHO guidelines for YF vaccines. The chimerisation process contributes to attenuation, as CYD-4 virus remained fully attenuated in cynomolgus monkeys, as assessed by low viraemia and absence of pathology, despite replacement of the entire YF 17D backbone with virulent YFV Asibi, which caused deaths/moribundity in controls.[23]

Attenuation of tetravalent CYD vaccine was also shown in the neurovirulence study in cynomolgus monkeys, where serum virus titres for both vaccines were below WHO guideline values for YF 17D vaccine. The potential for loss of attenuation is assessed in the section on genetic stability below.

Immunogenicity and protection

Three protection studies in NHPs were available. There is no standard nonclinical test for dengue protection, but a SC test dose of ~5$\log_{10}$CCID$_{50}$, with quantifiable viraemia, and challenge at least six months after vaccination, are common methods in published studies.

A published study in rhesus monkeys vaccinated once with monovalent CYD-2 at a dose of 2, 3, 4, or 5 $\log_{10}$ PFU and challenged SC 63 days later with 5 $\log_{10}$ PFU of a wt DENV-2 (S-16803) reported that all monkeys were protected against viraemia, and all except one monkey had a strong anamnestic response to challenge.[24]

Two monkey protection studies against wt DENV were submitted. The first study (Study SBi 1324-88) investigated protection against wt DEN viraemia induced after a mild SC challenge (4-5 $\log_{10}$ CCID$_{50}$) with each of the four wt DENV serotypes (DENV1-West Pacific 74, DENV-2 S16803 PDK-10, DENV-3-CH53489 PS, DENV-4 Carib. 341750) 6 months after a single immunisation of cynomolgus monkeys. The challenge viruses elicited peak levels and durations of viraemia in unvaccinated monkeys that were comparable to the tetravalent vaccine, suggesting that they were attenuated.

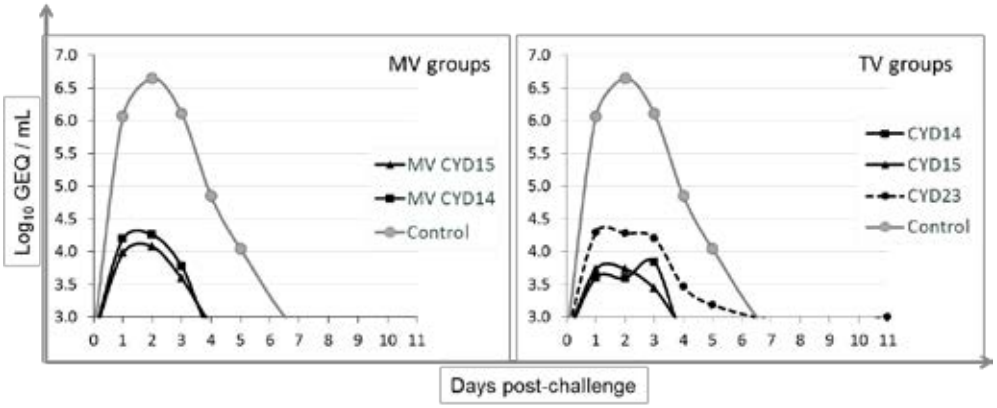
All monkeys raised neutralising antibodies post vaccination and 22/24 monkeys were fully protected (no viraemia). However, 1/6 vaccinated monkeys challenged by wt DENV-1, and 1/6 challenged by wt DENV-4 were partially protected, with viraemia lasting 4 days with a peak of 3.3 $\log_{10}$ PFU/mL in one monkey, and viraemia lasting 2 days with a peak of 1.7 $\log_{10}$ PFU/mL in the second. The two monkeys had low levels of neutralising antibody (PRNT$_{50}$ of 20 and 40 against wt DENV-1 and wt DENV-4, respectively) to the challenge virus, and did not become viraemic to CYD-1 or CYD-4 vaccine after vaccination. There was no enhancement of post-challenge viraemia in the 2 partially protected monkeys (peak viraemia in the 4 unvaccinated monkeys ranged from 3 to 3.4 PFU/mL for DENV-1, and 2.9 to 3.6 PFU/mL for DENV-4). Three of the protected monkeys had post-vaccination PRNT$_{50}$ of ≤ 20, which may indicate that cross-neutralising antibodies, and/or T-cell memory responses contributed to protection.

In the second study (Study DEN020Mk/C3), protection against a virulent DENV-2 (S16681) challenge was assessed using Phase II and Phase III vaccine lots (with vaccine virus titres of approximately 1 to 3 $\log_{10}$ CCID$_{50}$, administered SC), as part of the investigation of the discrepancy between immunogenicity and efficacy in clinical trial CYD23. Cynomolgus macaques were immunised twice, two months apart, with monovalent CYD-2 or tetravalent CYD and challenged with 7.0 $\log_{10}$ CCID$_{50}$ of DENV-2 by the IV route, 8 months after booster immunisation. CYD-2 was the weakest of the four CYD viruses in terms of ability to elicit neutralising antibodies and was not able to prevent DENV-2 infection efficiently under these stringent conditions. Only 2/18 monkeys were

[23] McGee CE, et al. Recombinant chimeric virus with wild-type dengue 4 virus premembrane and envelope and virulent yellow fever virus Asibi backbone sequences is dramatically attenuated in nonhuman primates. *Journal of Infectious Diseases* 197: 693-697 (2008).
[24] Guirakhoo F, et al. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. *J Virol.* 74: 5477-5485 (2000).

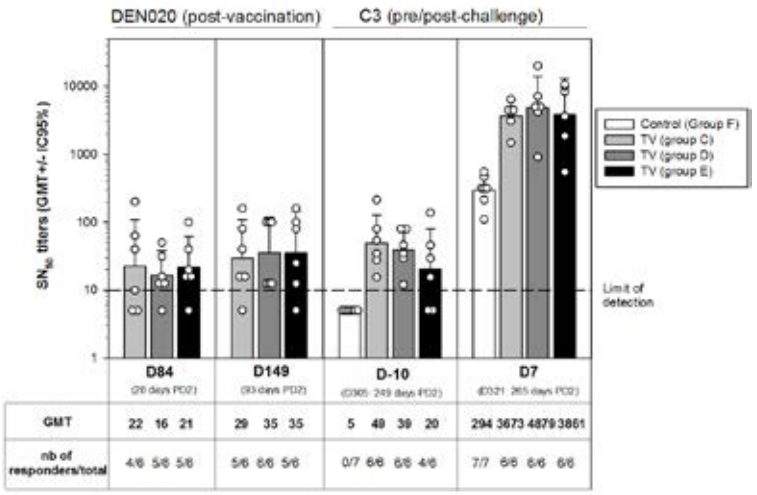
fully protected, that is, no detectable DENV-2. The remainder were partially protected in terms of viraemia reduced approximately 240-fold and more rapidly cleared (3 versus 5 days) (see following figure).

Figure 5: DENV-2 Viraemia after DENV-2 Challenge (Mean Titres per Group per Day) in cynomolgus monkeys vaccinated with monovalent (MV) or tetravalent (TV) vaccine



The vaccine elicited a strong anamnestic response (approximately 100-fold) in all monkeys, in the first week following challenge, and titres were significantly higher in the vaccine groups than in infected controls (following figure). Heterotypic responses to serotypes 1, 3 and 4 were also increased, but to a lesser extent than to serotype 2.

Figure 6: Pre- and post-challenge SN_{50} titres against DENV-2 in monkeys immunised with TV Lots #S4316 (Group C), #S4317 (Group D) or #S4233 (Group E)



From these studies it can be concluded that tetravalent CYD vaccine provided full protection to 22/24 of monkeys with a moderate SC challenge of wt DENV-1, 2, 3 or 4, and full protection in 2/18 monkeys, and partial protection in the rest challenged IV with a highly virulent DENV-2 strain.

Absence of viraemia accompanied by an anamnestic response after challenge has been observed with most,[25] but not all[26] live dengue vaccines. It is unclear whether the

[25] Sun W, et al. Protection of rhesus monkeys against dengue virus challenge after tetravalent live attenuated dengue virus vaccination. *J. Infect. Disease* 193: 1658-1665 (2006); Blaney JE, et al. Recombinant, live-attenuated tetravalent dengue virus vaccine formulations induce a balanced, broad and protective neutralising antibody response against each of the four serotypes in Rhesus monkeys. *J. Virol.* 79: 5516-5528 (2005);

anamnestic response was due to undetected virus replication, that is, non-sterilising immunity. It is also unclear whether sterilising immunity in NHPs is better correlated with protection in humans;[27] or reflects a more robust cell-mediated response (see below).

In the pivotal Phase III efficacy Studies CYD14 and CYD15, overall pooled data showed vaccine efficacy against symptomatic dengue disease due to any serotype was 60.3%, but efficacy varied with serotype, as low as 34.7% for serotype 2 in Study CYD14, and as high as 80.9% for serotype 4 in Study CYD15. A limited analysis showed that efficacy was higher in dengue-immune subjects than in non-immune subjects. The low overall efficacy of 30.2% in Phase IIb Trial CYD23, with inconclusive protection against serotype 2, despite neutralising antibodies in the same range against all 4 serotypes, prompted further nonclinical studies of potential causes (see Post-CYD23 investigations below).

Cross protection in vitro

Cross-neutralisation assessment was conducted between different strains and genotypes of the different DENV serotypes to determine the efficacy of the TV CYD DEN vaccine against circulating strains. These evaluations were done in vitro with sera of immunised monkeys or from clinical trials. In Studies CN0901 and CN1101, a panel of 82 wt isolates were used, representing approximately 20 strains per serotype, collected primarily during the last decade in 30 countries (Asia/Pacific and Latin America/Caribbean islands). The strains included the four serotypes and the majority of existing genotypes. Viruses were isolated and minimally amplified before evaluation against a pool of monkey sera generated after immunisation with the TV CYD DEN vaccine. No failure in neutralisation was observed against the geographically diverse strains with CYD DEN vaccine induced antibodies in monkeys. Studies CN1102 and CN1201 were conducted using pools of human sera from subjects included in the CYD28 (Asia) or in the CYD13 (Latin America) phase II trials collected after the second or the third immunisation. Each pool was composed of 8 to 10 sera having balanced PRNT titres against the 4 DENV serotypes, and six DENV were evaluated for each serotype: 2 prototype viruses (the parental DENV and the WHO reference DENV) and 4 recent field isolates (2003 to 2008), 2 from Asia, and 2 from Latin America. Both studies led to the same conclusion of a broad neutralisation of dengue strains from Asian and Latin America origins. Results showed that CYD DENV vaccine-induced antibodies in monkeys and humans were able to provide a broad coverage against geographically diverse strains of different genotypes, by cross-reacting against all collected strains for each of the four serotypes.

Post CYD23 investigations

In Phase IIb clinical Trial CYD23 (Ratchaburi, Thailand), limited and inconclusive protection against DENV-2 induced symptomatic disease was observed despite neutralising titres induced in the same range against all 4 serotypes in the $PRNT_{50}$ assay. Absence of correlation between pre-existing DENV-2 $PRNT_{50}$ and protection has also been previously reported;[28] and is reliant on what cell type and virus was used for the assay and to date a good in vitro correlate for protection has not been defined.

Briggs CM, et al. Live attenuated tetravalent dengue virus host range vaccine is immunogenic in African green monkeys following a single vaccination. *J. Virol.* 88: 6729-7642 (2014).

[26] Whitehead SS, et al. A live, attenuated dengue virus type 1 candidate with a 30-nucleotide deletion in the 3' untranslated region is highly attenuated and immunogenic in monkeys. *J. Virol.* 77: 1653-1657 (2003); Osorio JE, et al. Efficacy of a tetravalent chimeric dengue vaccine (DENVax) in cynomolgus macaques. *Am. J. Trop. Med. Hyg.* 84: 978-987 (2011).

[27] Sariol CA, White LJ. Utility, limitations, and future of non-human primates for dengue research and vaccine development. *Front Immunol.* 5:452 (2014).

[28] Endy TP, et al. Relationship of preexisting dengue virus (DV) neutralising antibody levels to viraemia and severity of disease in a prospective cohort study of DV infection in Thailand. *J Infect Dis.* 189: 990-1000 (2004).

CYD-2 virus deficiency

The sponsor provided data generated *in vitro* in MIM (MIMIC infection model which uses the ability of monocytes and dendritic cells from primary PBMCs to differentiate into immature and mature dendritic cells) assay to show that CYD-2 virus is less infectious than the 3 other serotypes and have suggested this could have an impact on its ability to elicit strong protective immunity. The hierarchy of serotype infectivity in MIM model cells showed that CYD-2 < CYD-4 (CYD-1 and CYD-3 intermediate) and this mirrored viraemia levels. Phase III efficacy studies (Study CYD14 (Asia) and Study CYD15 (Latin America) also showed efficacy against overall disease caused by DENV-2 was lower than that observed against the other serotypes.

Generally as discussed above, the immune response of tetravalent CYD vaccine differed between serotypes in monkeys, and DENV-2 immunogenicity was consistently low in all the monkey studies submitted in this application (Studies DEN011MK, DEN012Mk, DEN014Mk and DEN016Mk). The monkey studies and the clinical trials clearly demonstrate that the immunogenicity of tetravalent CYD for all 4 serotypes was variable, with serotype 2 the weakest in humans and serotypes 2 and 3 the weakest in monkeys. Studies with monovalent CYD have shown absence of viraemia when CYD-2 was administered alone in monkeys and immunogenicity of monovalent CYD-2 was either low or absent. In post-CYD23 studies, neither monotypic, heterotypic nor multiplicity immunity against DENV-2 disease was achieved. Incomplete heterotypic and multitypic immunity in these post-CYD23 studies have been suggested to be due to absence of strong protective dengue T cells responses which requires DENV NS1.[29]

The sponsors ruled out possibility of interference in the tetravalent vaccine by suggesting CYD serotypes when tested individually in a MIM assay showed no competitive interference since the same hierarchy pattern was observed as that of the tetravalent CYD vaccine CYD-4 >> CYD-3 > CYD-1 > CYD-2. Since failure of symmetrical production of neutralising antibodies to each of the four DENV has been reported for all live- attenuated viruses given as mixtures in monkeys, interference cannot be completely ruled out however studies with monovalent vaccines suggest CYD-2 is less infectious and hence less immunogenic and therefore could be responsible for limited protection in this trial.

Potential of new DENV lineage not to be captured by tetravalent CYD vaccine

The sponsors have also provided evidence of a new lineage of DENV-2 circulating in Thailand, at the time of the clinical trial. They have provided in vitro seroneutralisation results indicating responses induced by the tetravalent CYD vaccine were capable of neutralising parental and wt strains (n = 7) circulating in the CYD23 trial area, including DEN-2 strains to the same extent as other serotypes. However, as discussed above, neutralising antibodies measured in non Fc receptor cells have been shown not to be a good correlate of protection. To date a good in vitro correlate for protection has not been defined and therefore poor efficiency in neutralising ability of CYD-2 antibodies in vivo cannot be ruled out. The use of a Fc-receptor-bearing cell system as an alternative to classical neutralisation tests to study vaccine-induced immunity may help identify an immune correlate.[30]

Potential of host/immune response against DENV-2 to enhance infection by this serotype

The sponsor provided an *in vitro* study to show that serum from neutralising antibody titres in FcγRIIa + CV1 cells from human volunteers that received the tetravalent CYD vaccine did not enhance DENV-2 activity. However, FcγRIIa CV1 cells (transfected monkey kidney derived cell line) are limited in their capacity to mimic the physiological

[29] Halstead SB. Identifying protective dengue vaccines: Guide to mastering an empirical process. *Vaccine* 31: 4501-4507 (2013).
[30] Moi MI, et al. Efficacy of tetravalent dengue vaccine in Thai schoolchildren. *Lancet* 381:1094 (2013).

co-expression of both FcγRIIa and FcγRIIb found in DEN targets cells. FcγRIIa (facilitates DENV infection) and FcγRIIb (inhibits the infection).[31] Therefore it would be more appropriate to use cell lines that have both receptors, where the competition for binding of available DEN immune complexes and resultant neutralisation and or enhancement of dengue infection could be more accurately observed. These studies with appropriate models would also add value to track long-term efficacy and safety of the vaccine.

Overall conclusion for post CYD23 studies

These investigations emphasised the complexity of the mechanisms involved in protective immunity and highlighted the possibility of low infectivity of CYD-2 (presumably inherent to the virus) and lack of immunological potency of CYD-2 (despite high anti DENV-2 neutralising antibody levels) to be the main factors that could explain the outcome of this Phase IIb study.

Potential of CYD vaccine to sensitise to secondary DENV infection

In humans, more severe dengue disease may occur upon a secondary heterotypic DENV infection. A wt dengue infection in an individual before completion of the 3 dose vaccine regimen, prior to a full immune response to all 4 serotypes, may pose a theoretical risk of sensitisation to secondary infection. Furthermore, in an endemic area, many individuals may be seropositive to dengue prior to vaccination, or have prior YF 17D immunisation, with a theoretical risk of enhanced vaccine viraemia. Published data on enhancement of wt dengue infection in NHPs are limited. Enhanced viraemia has been reported in rhesus monkeys upon secondary experimental infection with DENV-2, but not DENV-1, 3, or 4.[32] However, enhanced viraemia was not observed in cynomolgus monkeys after sequential exposure to DENV-1 or 4 followed by DENV-3, then a year later by DENV-4.[33]

The potential for a more severe dengue infection after CYD vaccination was assessed in the primary pharmacology studies in terms of enhancement of viraemia, as NHPs do not develop the clinical symptoms of dengue disease seen in humans. In the dengue SC challenge study in cynomolgus monkeys, no enhanced viraemia was observed in the unprotected animals which had low homotypic antibodies against the infecting serotype (Study SBi 1324-88). Sequential heterologous bivalent vaccination was also not associated with enhanced viraemia (Study DEN011Mk), and monkeys immunised with tetravalent CYD vaccine after YF17D vaccine also did not show increased viraemia in comparison to a CYD priming and boost regimen. However the validity of the NHP model is unclear, since enhancement of viraemia due to secondary heterologous wt dengue infection, which has been reported for serotype 2 in rhesus monkeys, has not been observed in cynomolgus monkeys (see above). It was also unclear if the time between last vaccination and challenge was sufficient to exclude the presence of transient cross-protection. In a response to TGA questions, regarding the validity of the NHP model, the sponsor has stated that severe dengue disease in humans has a multifactorial origin and the predominant role of ADE in this regard has been questioned in some studies.[34] The sponsor states absence of ADE/sensitisation observed in different studies in monkeys does

[31] Boonnak K, et al. Human FcgammaRII cytoplasmic domains differentially influence antibody-mediated dengue virus infection. *J Immunol.* 190: 5659-65 (2013); Chan KR, et al. Ligation of Fc gamma receptor IIB inhibits antibody-dependent enhancement of dengue virus infection. *Proc Natl Acad Sci USA.* 108: 12479-84 (2011).

[32] Halstead SB, et al. Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *J Infect. Dis.* 128: 15-22 (1973).

[33] Koraka P, et al. Characterization of humoral and cellular immune responses in cynomolgus macaques upon primary and subsequent heterologous infections with dengue viruses. *Microbes and Infection* 9: 940-946 (2007).

[34] Laoprasopwattana K, et al. Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viraemia in secondary DV infection. *J Infect Dis.* 192: 510-519 (2005); Libraty DH, et al. A prospective nested case-control study of Dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. *PLoS Med.* 6: e1000171 (2009).

not necessarily lack predictive value. Clinical studies would need to be closely monitored to determine the cross enhancing potential and related complications of this vaccine. In clinical trial CYD14 an increase in hospitalisations was observed in the youngest age group, 2 to 5 years, suggestive of an enhanced risk in seronegative individuals, raising the possibility that CYD vaccine might act like a natural infection and increase the risk of severe disease upon first natural dengue infection. The indicated age range for CYD vaccine was limited to 9 years or more as a precaution.

Recent research published after the date of the submission has also raised the possibility of cross-interactions between Zika virus (ZIKV) and DENV. ZIKV differs from DENV by around 41 to 46% in the sequence of the envelop protein, recent studies have shown the similarities are sufficient to allow cross-reaction of antibodies to DENV with ZIKV, resulting in potential ADE enhancement of infection.[35] Though enhancement of ZIKV infection due to DEN antibodies in humans or in animal models have not been observed to date and may only occur in vitro and not in vivo as seen with other flaviviruses, for example, West Niles virus, this still warrants investigation. In a Section 31 response, the sponsor has provided reference to ongoing research on Zika viraemia in dengue immune monkeys where preliminary studies in animals pre-exposed to DENV neutralisation titres did not correlate with increase in viraemia. Other infectious flaviviruses in Australia are Murray River encephalitis and Kunjin viruses, there were no nonclinical data on cross-reactivity with these viruses. Hence there is also a theoretical risk of sensitisation with these viruses.

Genetic stability

Reversion to virulence (mutations during manufacture, in vivo)

CYD DENVs have been made on YF-17D backbone for which attenuation-related mutations have been well mapped and identified in comparison to the sequence of YF-17D and related vaccines with the wild type Asibi parental strain.[36] The 17D and Asibi strains differ at around the 48 to 68 nucleotide positions scattered throughout the genome, resulting in around 22 to 32 amino acid differences. Therefore reversion event to virulence is highly unlikely as it would require a large number of back mutations.

GMP lots of CYD DENVs were sequenced at each step of the manufacturing process i.e. Pre-Master Seed Lot (PMSL) (P8) to Master Seed Lot (MSL) (P9), Working Seed Lot (WSL) (P10), at the Drug Substance (DS) stage (P11), and at late passages beyond DS stage (P21) and showed limited variations. Absences of major viral sequence changes were also observed in the manufacturing changes between phase I, II and III vaccines. These results indicate the CYD DEN viruses amplified in vero cells to produce tetravalent CYD vaccine are genetically stable.

In vivo genetic stability experiments in mosquitoes showed that CYD DENV poorly infect and replicate in *Aedes aegypti* midgut tissue via an artificial infectious blood meal. Therefore mosquitoes had to be intrathoracically inoculated with CYD DENV and genomic sequences of the prM and E gene regions of the CYD DENV isolated, from these mosquitoes showed no nucleotide differences from the seed virus in any of the CYD DEN-1, 2, 3 and 4 viruses.[37] Cynomolgus monkeys were vaccinated with MV formulations of CYD DENVs and the isolated viral plaques (1/3 monkeys inoculated with CYD-1, and 3/3 monkeys

[35] Dejnirattisai W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat Immunol.* 17: 1102-1108 (2016).
[36] Hahn CS, et al. Comparison of the virulent Asibi strain of yellow fever virus with the 17D vaccine strain derived from it. *Proc Natl Acad Sci USA.* 84:2019-23 (1987); Dos Santos CN, et al. Complete nucleotide sequence of yellow fever virus vaccine strains 17DD and 17D-213. *Virus Res.* 35: 35-41 (1995).
[37] Johnson BW, et al. Analysis of the replication kinetics of the ChimeriVax-DEN-1, 2, 3, 4 tetravalent virus mixture in Aedes aegypti by real-time reverse transcriptase-polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 70: 89-97 (2004).

inoculated with either CYD-3 or CYD-4) were amplified and sequenced. 3 mutants (in CYD-1 and 3) were identified, CYD-3 mutations were inconsequential in terms of neurovirulence for suckling mice. However, mutations in the CYD-1 chimera, reverted to neurovirulence of suckling mice similar to that of their pre-PMS virus. Nevertheless, all viruses isolated from monkeys remained significantly less neurovirulent than the control YF-17D vaccine. In humans, CYD DENV isolated from clinical trial patients are generally limited as viraemia levels are generally not detectable. Six CYD DENV isolates from clinical trial (CYD11) patients were either identical to the reference strain (4/6) or were synonymous mutants (2/6).

A major concern for production of live attenuated viral vaccines is that the attenuated phenotype may revert upon serial passaging in vivo. Such concerns appear minimal in the case of tetravalent CYD DEN vaccine due to the number of mutations required for reversion and the relatively high fidelity of the RNA polymerase encoded by CYD vaccine. Consistent with these predictions it can be concluded that CYD vaccine is genetically stable in vitro as seen throughout the vaccine production chain. However, in vivo, particularly in monkeys, neurovirulent mutations were identified but as they were less neurovirulent than the control YF-17D which is not neurovirulent in humans, it was considered safe. Even in the unlikely event that the mutated virus could escape the host immune response and cross the blood barrier to infect the brain parenchyma, it is not likely to cause pathology or clinical symptoms.

Recombination with circulating flavivirus

Studies have shown flaviviruses have low propensity for both homotypic and homologous recombination.[38] Recombination events in these studies could be quantified and showed limited recombination using YF-17D virus as a model. Worst case scenarios for chimeras constructed were highly attenuated compared to their parental viruses and in an unlikely event of recombination or substantial backbone reversion, hypothetical designed worst-case virulent recombinant sequences, showed no enhancement of transmissibility of TV CYD DENVs in *Aedes aegypti* mosquitoes or increase vertebrate pathogenicity.[39] Super infection resistance have also been demonstrated with flaviviruses which makes co-infection in vivo even less likely. Given that only low level viraemia has been observed after tetravalent CYD vaccination in human volunteers, coupled with low mosquito infectivity, the risk of mosquito infection and transmission of CYD recombinant/revertant viruses in nature is considered very unlikely.

Safety pharmacology

Neurovirulence study in cynomolgus monkeys

Flaviviruses are associated with neurotropic and viscerotropic diseases;[40] and vaccination with YF 17D vaccines is also associated with rare and acute viscerotropism and neurotropism diseases.[41] Therefore, neurotropic and viscerotropic diseases were pertinent in assessing the safety of this vaccine. DENV is not normally a neurotropic virus. Although encephalopathy is a common neurological complication of dengue fever, it is

[38] Taucher C, Berger A, Mandl CW. A trans-complementing recombination trap demonstrates a low propensity of flaviviruses for intermolecular recombination. *J. Virol.* 84: 599-611 (2010); McGee CE, et al. Stability of yellow fever virus under recombinatory pressure as compared with chikungunya virus. *PLoS One.* 6: e23247 (2011).

[39] McGee CE, et al. Recombinant Chimeric Virus with Wild-Type Dengue 4 Virus Premembrane and Envelope and Virulent Yellow Fever Virus Asibi Backbone Sequences Is Dramatically Attenuated in Nonhuman Primates. *J Infect. Dis.* 197: 693-7 (2008); McGee CE, et al. Substitution of Wild-Type Yellow Fever Asibi Sequences for 17D Vaccine Sequences in ChimeriVax-Dengue 4 Does Not Enhance Infection of Aedes aegypti Mosquitoes. *J Infect. Dis.* 197: 686-92 (2008).

[40] Burke DS, Monath TP. Flaviviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA,Martin MA, Roizman B, Straus SE, eds. Fields Virology, 4th ed. Philadelphia: Lippincott Williams and Wilkins, 2001; 1043-1125.

[41] Lindsey NP, et al. Adverse event reports following yellow fever vaccination. *Vaccine* 26: 6077-6082 (2008).

usually secondary to shock, hepatitis, coagulopathy, and concurrent bacterial infection. Neurotropism was evaluated in the repeat dose toxicity study and the biodistribution study (discussed in the appropriate sections) using the clinical dose and route in monkeys, and no sign of neurotropism was detected (that is, no virus was detected in the nervous tissues and no histopathology indications were observed).

The WHO guidance for live dengue vaccines recommends a test for neurovirulence in nonhuman primates via the IC route. Neurovirulence was assessed by inoculating Phase I tetravalent CYD DEN (5 $\log_{10}$ $CCID_{50}$) via the IC route in adult cynomolgus monkeys (11/sex), followed by a 30 day observation period. The testing of tetravalent CYD rather than the individual serotypes;[42] was based on published neurovirulence data in suckling mice which showed a lack of interference between serotypes.[43]

The study design was based on the monkey safety test for the Yellow fever vaccines;[44] as recommended by the WHO, and the tetravalent CYD DEN vaccine was compared to a YF 17D vaccine (YF-VAX), used as the reference vaccine, since its degree of neurovirulence is well established as per requirements for yellow fever vaccine monkey safety testing. Histopathological findings with the IC route showed the neurovirulence of tetravalent CYD vaccine was lower than that observed with the YF 17D vaccine (which is not neurovirulent in humans) as shown by comparable scores for clinical signs of encephalitis and statistically lower scores for neuro histologic evaluation in monkeys given tetravalent CYD vaccine than in animals given YF 17D vaccine ($p < 0.01$). Therefore, these results indicate, tetravalent CYD vaccine's neurotoxic profile is acceptable.

The levels of viraemia load were low in the neurovirulence study. In tetravalent CYD vaccine-treated monkeys, all 11 monkeys become viremic, and peak serum virus titre ranged from 50 to 2000 PFU/mL. In the YF-Vax group, peak serum virus titres ranged from 20 to 860 PFU/mL. Strain-specific titration showed that peak titres ranged from 10 to 10, 10 to 50, 30 to 1220 and 10 to 1610 PFU/mL for CYD serotypes 1, 2, 3 and 4, respectively. Monkey viraemia titres were below 500 and 100 mouse IC LD_{50} values (estimated to approximately equal to 20,000 and 4,000 Vero cell PFU/0.03 mL, respectively, for YF-Vax), which are the maximum acceptable titres for individual monkey and group (that is, present in no more than 10% of the monkeys), as established under the WHO requirements for yellow fever 17D vaccine in the viscerotropism test section.[45] The levels of viraemia, virus distribution data and the histopathology examination, indicates that viscerotropism is unlikely with CYD vaccine.

Pharmacokinetics

Distribution, persistence and shedding

DENV entry into host cells is mediated by the DEN E protein, specific human cell receptors for DENV are unknown, although a number of putative receptors have been proposed.[46] Basal keratinocytes, Langerhans cells, dendritic cells, macrophages, monocytes,

[42] WHO (2013). Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). WHO Expert Committee on Biological Standardization. WHO Technical Series No. 979, Annex 2.
[43] Guirakhoo F, et al. Safety and efficacy of chimeric yellow fever-dengue virus tetravalent vaccine formulations in nonhuman primates. *J. Virol.* 78: 4761-4775 (2004).
[44] Requirements for Yellow Fever Vaccine. Geneva: World Health Organization; Annex 2 (WHO Technical Report Series, No. 872) 1998; Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live,attenuated). WHO Expert Committee on Biological Standardization. WHO Technical Report Series No. 979, Annex 2. 2013.
[45] Robert E, et al. Exposure to yellow fever vaccine in early pregnancy. *Vaccine* 17: 283-285 (1999).
[46] Cruz-Oliveira C, et al. Receptors and routes of dengue virus entry into the host cells. *FEMS Microbiology Reviews* 39: 155-170 (2015).

hepatocytes and other cell types have been reported as viral targets. Cell uptake of DENV by Fc receptors is a postulated mechanism of severe dengue infection after heterologous secondary infection.

Phase III lot tetravalent CYD vaccine was evaluated in a monkey biodistribution and shedding study (Study SP0056 BD1001) to assess the distribution, the persistence or elimination, as well as the shedding of dengue vaccine. Cynomolgus monkeys (15/sex) received a full human dose of 5 $\log_{10}$ $CCID_{50}$ (0.5 mL) of tetravalent CYD vaccine SC followed by a 3, 9 or 21 day observation period.

At the end of each observation period, a range of organ and tissues were taken, weighed and processed for qRT-PCR analysis for YF NS5, and microscopic examination. The CYD dengue vaccine RNA was transient and limited to the injection site tissues, the lymphoid tissues, spleen and/or the liver, distant lymph nodes, thymus, adrenals, bone marrow and skeletal muscle. On Day 22 post-dose it was only detected in the injection site tissues and draining lymph nodes in a few animals but not in any other tissue. There was no RNA detected in the nervous system tissue at any time point and no relevant changes, including changes in liver enzyme activity level in blood or microscopic findings at the histopathological examination. Tetravalent CYD vaccine was not detected in urine, faeces, injection site swab and saliva samples on Days 4, 10 and 22.

In humans, wt DENV has been detected in urine and saliva after infection;[47] and YF vaccine virus shedding in human urine has been reported.[48] CYD vaccine virus shedding in urine and saliva was investigated in a small number of vaccinated subjects in one Phase I clinical study (CYD04, 11subjects) and in a larger subset in Phase III Study CYD17 (95 subjects). Virus shedding was observed in 2 subjects at levels close to the LLOQ. No replication competent virus was detected in these samples.

Animal to human dose ratios (mg/kg)

In most studies, the full human dose was used (Table 8) as per WHO guidelines on Nonclinical Evaluation of Vaccines. Dose ratios on mg/kg basis are tabulated below.

Table 8: Dose ratios on mg/kg basis

Study Type	Species	Dose	Approximate animal: human dose ratio (mg/kg)
Neurovirulence study (Study T-100-001)	Cynomolgus monkey	5 log10 TCID50	17
Biodistribution study (Study SP0056 BD1001)	Cynomolgus monkey	5 log10 CCID50	17
Dose range studies in mice (Study SP0056 PS1003)	Mice	5, 6.5 and 8 log10 CCID50	1700, 54400 and 1700000
Developmental and reproductive toxicity study in mice (Study SP0056 DV1014)	Mice	5, 6.5 and 8 log10 CCID50	1700, 54400 and 1700000
Lactation study in mice (Study SP0056 DV1109)	Mice	5, 6.5 and 8 log10 CCID50	1700, 54400 and 1700000

[47] Poloni TR, et al. Detection of dengue virus in saliva and urine by real time TR-PCR. *J. Virol.* 7: 22 (2010).
[48] Domingo C, et al. Detection of yellow fever 17D genome in urine. *J. Clin. Microbiol.* 49: 760-762 (2011).

Study Type	Species	Dose	Approximate animal: human dose ratio (mg/kg)
Dose range studies in rabbits (Study SP0056 PS1002)	Rabbit	5, 6.5 and 8 log10 CCID50	14, 448, and 14000
Developmental and reproductive toxicity study in rabbits (Study SP0056 DV1013)	Rabbit	5 log10 CCID50	14
Female mouse, female rabbit, cynomolgus monkey and adult human bw~30 g, 3.5 kg, 3.0 kg and 50 kg respectively.			

Toxicology[49,50]

Acute toxicity

Nonclinical safety of tetravalent CYD vaccine after a single dose injection was evaluated as part of the repeat dose toxicity study, and the single dose biodistribution and toxicity study, in cynomolgus monkeys.

Repeat-dose toxicity

One repeat dose toxicity study (Study RQH00006) was submitted that evaluated both the systemic and local toxicity of phase II tetravalent CYD vaccine in the cynomolgus monkey. Two groups of 3 male and 3 female naïve cynomolgus monkeys were given three SC injections (clinical route) at approximately 4-week intervals (the recommended clinical dose administration is 3 times at 6 months intervals) of tetravalent CYD vaccine at 5 $\log_{10}$ $CCID_{50}$ of each serotype in 0.5 mL (which is around 17 times the human dose based on $CCID_{50}$/kg body weight). The study was consistent with WHO nonclinical guidelines;[3] in terms of administration of 3 consecutive human doses by the clinical dose route, and demonstrated immunogenicity in the test species.

Potential treatment-related effects were evaluated after either a 10 or 21 day observation period. No premature deaths, adverse clinical signs and treatment-related changes in body temperature, body weight or food consumption, vaccine-related local reactions at the injection site, ophthalmological findings, vaccine-related changes in clinical pathology and urinalysis parameters were noted. There were also no changes in organ weights and no vaccine-related macroscopic or microscopic findings.

[49] Careful consideration of the doses used in nonclinical studies is necessary to fulfil the scientific needs of safety assessment and to satisfy regulatory authorities. The current Committee for Proprietary Medicinal Products (CPMP) note for guidance on repeated dose toxicity studies indicates that doses should be selected to establish a dose or exposure response to treatment. This can generally be achieved by the use of three groups of animals receiving the test item, at low, intermediate and high doses, plus a control group which receives vehicle alone. Experience has shown that three doses will usually cover the span between no effect and adverse effects although there are exceptions. The CPMP guidance also indicates that the high dose should be selected to enable identification of target organ toxicity, or other non-specific toxicity, or until limited by volume or limit dose. In addition to establishing toxicity, it is necessary from a scientific perspective to establish the No Observed Effect Level (NOEL) and/or the No Observed Adverse Effect Level (NOAEL) that may be used along with other information, such as the pharmacologically active dose, to determine the first dose in human studies.

[50] LD=low dose, MD=middle dose and HD=high dose used in a particular nonclinical study.

The primary pharmacology studies in monkeys included observations of clinical signs, bodyweights, food consumption, and in some cases serum chemistry and haematology, with no adverse findings, although the results were not reported in all studies. The biodistribution study in which monkeys were administered the human dose included toxicological observations (clinical signs, local reactions, bodyweights, rectal temperatures, haematology, biochemistry, urinalysis, organ weights, macroscopic and microscopic pathology). The only treatment-related finding was occasional transient and minimal erythema at the injection site, correlated with a minimal to slight inflammatory reaction.

In conclusion, the vaccine was well tolerated at the appropriate dose tested when administered in repeated SC inoculations. The studies were in accordance with WHO Guidelines on Nonclinical Evaluation of Vaccines and the ICH Harmonised Tripartite Guideline S6 'Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals'.

Genotoxicity and carcinogenicity

Genotoxicity and carcinogenicity studies were not required as per WHO and EMA guidelines.

Reproductive toxicity

Wild-type YF epidemiology

YFV infection in humans is marked by an initial period of viraemia, and 'viscerotropic' infection of the liver, spleen, heart and kidneys. The ensuing haemorrhagic disease may be fatal. The virus may also be 'neurotropic', causing encephalitis. Spontaneous abortion, stillbirth, and congenital malformation have not been observed after yellow fever epidemics.

YF vaccine and pregnancy

A number of studies have investigated YF vaccine exposure during inadvertent pregnancy.[51] The studies have reported several hundred pregnancies, mainly during mass immunisation campaigns. They all reported rates of abortion, stillbirths and major fetal malformations within normal population ranges.

Wild-type dengue

The information on the possible adverse consequences of DEN infection in pregnancy is limited.[52] DEN virus is teratogenic in humans and can be vertically transmitted to fetuses.[53] Maternal consequences of DEN infection include premature labour, haemorrhage during labour and retroplacental haematoma.[61] Fetal consequences of DEN

[51] Tsai TF, et al. Congenital yellow fever virus infection after immunization in pregnancy. J Infect Dis. 168: 1520-1523 (1993); Nasidi A, et al. Yellow fever vaccination and pregnancy: a four-year prospective study. *Trans. R. Soc. Trop. Med. Hyg.* 87: 3379 (1993); Robert E, et al. Exposure to yellow fever vaccine in early pregnancy. Vaccine 17: 283-285 (1999); Suzano CES, et al. The effects of yellow fever immunization (17DD) inadvertently used in early pregnancy during a mass campaign in Brazil. *Vaccine* 24: 1421-1426 (2006); Cavalcanti DP, et al. Early exposure to yellow fever vaccine during pregnancy. *Tropical Medicine and International Health* 122: 833-837 (2007).

[52] Paixao ES, et al. Dengue during pregnancy and adverse fetal outcomes: a systematic review and meta-analysis. *Lancet Infectious Diseases* 16: 857-865 (2016).

[53] Basurko C, et al. Eur. J. Obstet. Gynecol. Reprod. Biol. 147: 29-32 (2009); Carles GH, Peiffer Talarmin A. Effects of dengue fever during pregnancy in French Guiana. *Clin. Infect. Dis.* 28: 637-40 (1999); Perret C, et al. Dengue infection during pregnancy and transplacental antibody transfer in Thai mothers. *J. Infect. Dis.* 51: 287-93 (2004); Chye JK, et al. Vertical transmission of dengue. *Clin. Infect. Dis.* 25: 1374-7 (1997); Tan PC, et al. Dengue infection in pregnancy. Prevalence, vertical transmission, and pregnancy outcome. *Obstetrics & Gynecology* 111: 1111-1117 (2008).

infection include fetal death in utero, late miscarriage, acute fetal distress during labour, maternal fetal transmission and neonatal death.[61]

Submission for reproductive studies

The sponsor submitted two 'model' investigate studies in non-pregnant female rabbits and mice (SP 0056 ISO906 and SP0056 ISO907, respectively) , two preliminary dose-range studies in pregnant female rabbit and mice (SP0056 PS1002 and SP0056 PS1003, respectively) two pivotal developmental and reproductive toxicity studies which included a developmental and reproductive toxicity study in rabbits (SP0056 DV1013) and an embryofetal toxicity study in mice (SP0056 DV 1014) designed in compliance with ICH Harmonised Tripartite Guideline S5 (R2) and S6 and a lactation study in mice (SP0056 DV1109).

Animal model investigate studies

The rabbit and mouse 'model' investigative studies were conducted using the clinical dose of Phase II tetravalent CYD vaccine *i.e.* 5 $\log_{10}$ $CCID_{50}$ (SC and IV), which is ~17x the human dose in rabbits and ~1700 times in mice) or high dose CYD vaccine bulks at doses of 9 $\log_{10}$ $CCID_{50}$(IV). Viraemia was detected at low levels on the day after the injection in rabbits given the high dose via the IV route, however all rabbits seroconverted to all serotypes whatever the route of injection and the dose level, therefore the rabbit was selected by the sponsors for the evaluation of the effects of the antibody response, but not the viraemia. In contrast, viraemia was detected in all mice given the high dose by the IV route, and seroconversion was limited to a few animals, making the mouse model suitable for the evaluation of the effects of the viraemia, but not the antibody response. Both species have their limitations (rabbit does not show viraemia and mouse does not show immunogenicity) and therefore the results in the pivotal studies will not fully replicate the clinical situation.

Dose finding studies

In the mouse study, dose levels of 5, 6.5 and 8 $\log_{10}$ $CCID_{50}$ (approximately 1,700 x, 54,400 x and 1,700,000 x the human dose on a mg/kg basis) were selected based on the responses elicited by administration of tetravalent CYD at the different doses. Briefly the dose level of around 5 $\log_{10}$ $CCID_{50}$ had no effects in dams and fetuses, no antibody or virus detection, 6.5 $\log_{10}$ $CCID_{50}$ had no effects in dams and fetuses, limited antibody response and transfer, and no virus detection. However at 8 $\log_{10}$ $CCID_{50}$, virus was detected in fetuses with limited antibody response and some maternal toxicity and developmental toxicity were observed (i.e. reduced maternal body weight and food consumption, reduced fetal body weight and an increase in average number of resorptions per litter).

Dose finding studies in rabbits concluded the human clinical dose of 5 $\log_{10}$ $CCID_{50}$ (around 14 x the human dose on a mg/kg basis) is appropriate as higher doses tested (that is, up to 8 $\log_{10}$ $CCID_{50}$) generally showed similar no treatment related effects. However, it is worth noting that in the 8 $\log_{10}$ $CCID_{50}$ dose, fetal anomaly (meningocele and eye lid open) was observed in 1 of the 46 fetuses examined.

The test doses meet the WHO guideline criteria, however deficiency of viraemia or immunogenicity in the animal models tested limits these studies finding as it does not fully replicate the clinical situation. The sponsor decided not to use nonhuman primates due to ethical and technical issues, balancing the need for species established as models for developmental and reproductive toxicity (DART) studies, a detectable viraemia, a measurable immune response to the vaccine, and exposure of fetuses to both virus and antibodies.

Pivotal developmental, reproductive toxicity (DART) and embryofetal toxicity studies

In the pivotal embryofetal toxicity study, after mating, 25 female mice were administered one single IV dose of around 5, 6.5 or 8 $\log_{10}$ $CCID_{50}$ (each serotype, up to around 1700,

54400 and 1700000 x the human dose on a mg/kg basis) of Phase III tetravalent CYD vaccine on gestation Day (GD) 6, 9 or 12 (5 controls and 25 treated mice per time point in the main study). Clinical observations were recorded routinely till GD 18, when mice were euthanised, for ovarian uterine examination, and immunology investigations. CYD vaccine showed no teratogenic potential at any doses in mice. Reductions in maternal body weight gains and food consumption occurred in mice given a dose of 6.5 or 8 $\log_{10}$ $CCID_{50}$ on GD 6, 9 or 12. Post implantation loss was increased in females given a dose of 6.5 or 8 $\log_{10}$ $CCID_{50}$ on GD 6 or 9. Fetal body weights were reduced in litters of females given 8 $\log_{10}$ $CCID_{50}$ on GD 9 or 12. They were significant differences in fetal ossification site averages between groups and the most pronounced differences were observed in litters dosed on GD 9 and were observed in each vaccine treated group. Reduced skeletal ossification occurred in litters of females given 5 $\log_{10}$ $CCID_{50}$ on GD 9 or 12, but was not considered to be of any toxicological significance because the reductions were minimal, occurred in the absence of reduced fetal body weights, fetal abnormalities, effects on maternal body weight or food consumption, and were predicted to most likely resolve itself with further growth and development. Reduced skeletal ossification in litters of females given 6.5 $\log_{10}$ $CCID_{50}$, where maternal body weight or food consumption were observed in absence of reduced fetal body weights and fetal abnormalities were also suggested to resolve themselves. However, in the 8 $\log_{10}$ $CCID_{50}$ group, reduced skeletal ossification occurred with reduction in fetal body weights and therefore it was concluded that it is a treatment related effect at this high dose.

DENV RNA was detected in 12/13 dams given the high dose but not in dams given the low or mid dose, and no virus was detected in the embryos in mice. There was a dose-related increase in CYD vaccine antibodies in dams, and low antibody transfer to fetuses on GD 18 at all doses and schedules of injection.

In the pivotal DART study, 55 female rabbits were IV administered Phase III 5 $\log_{10}$ $CCID_{50}$ of tetravalent CYD vaccine, 30 and 10 days before mating and 6, 12 and 27 days after mating. At the clinical dose (~14x the human dose on a mg/kg basis), no adverse effects on the mating performance and fertility of the vaccinated rabbit, no teratogenic potential and no effect on pre- and post-natal development in the rabbits were noted. Adequate exposure was demonstrated by presence of anti-CYD antibodies against all serotypes in the serum of all treated rabbits, which were transferred to the fetuses and pups. No treatment related effects were seen on F^1 pup survival, growth and development.

These studies showed no potential developmental and female reproductive effects from the clinical dose of the vaccine in rabbits (at 14 x the human dose) and embryofetal toxicity studies in mice (at 1700 x the human dose).

Although pregnancy was an exclusion criterion during all vaccine clinical trials, the vaccine was inadvertently administered to females who were not aware of their pregnancy or who became pregnant shortly after. A total of 404 pregnancies were reported with the vaccine, 341 were unexposed, 36 were exposed but not yet pregnant, 22 were exposed and pregnant, and exposure could not be determined for 5 pregnancies. Among the 22 females who were exposed to the vaccine during pregnancy, 3 had an adverse pregnancy outcome: death in utero, stillbirth and blighted ovum. In all cases important risk factors were identified. Compared to placebo, no difference between the 2 groups was observed in adverse pregnancy outcomes.

In view of the limited nonclinical and clinical data, and pregnancy data on wt DEN, contraindication of the vaccine in pregnancy is appropriate (PI). The live attenuated YF vaccine Stamaril and the JE vaccine Imojev are both contraindicated in pregnancy.

The PI statement that women of childbearing age should be advised not to become pregnant for 4 weeks after receiving any injection of Dengvaxia is appropriate, and consistent with the maximum duration of viraemia observed in monkeys and humans, and

biodistribution data in monkeys. The WHO position paper on CYD-TDV vaccine states: *'Women of child-bearing age who are targeted for vaccination do not need to be tested for pregnancy.'*

Australian pregnancy classification

The sponsor has proposed Australian Pregnancy Category B2.[54] This category is appropriate even though studies in rabbits and mice showed no adverse effects when the clinical dose of tetravalent CYD vaccine was administered. Rabbits and mice showed the limitations of not being either viremic or immunogenic as discussed above. The YF vaccine Stamaril and the JE vaccine Imojev both have a pregnancy category of B2.

Lactation study

One IV injection of 5, 6.5 or 8 $\log_{10}$ $CCID_{50}$ of tetravalent CYD vaccine (which is approximately 1700 x, 54400 x and 1700000 x the human dose on a mg/kg basis) on Day 14 of lactating mice (25 mice per treatment group) was well tolerated with treatment-related effects limited to a transient body weight loss on the day after injection in females given 6.5 and 8 $\log_{10}$ $CCID_{50}$ and no treatment-related changes in litter parameters at any dose. Exposure to tetravalent CYD vaccine virus was shown by detection of the virus in approximately 6 out 15 satellite mice given 8 $\log_{10}$ $CCID_{50}$ with no evidence of viral transfer to pups. Seroconversion of at least one serotype was observed in majority of the dams and anti-CYD antibody transfer from dams to pups was seen in > 50% of the pups. Due to the limitation of this model (that is, absence of immunogenicity in the mouse model) the results of this study cannot be fully applied to the clinical situation.

In humans, a possible occurrence of dengue virus transmission via breast milk has been reported.[55] Dengvaxia is appropriately contraindicated in breastfeeding women (PI).

Local tolerance

Local tolerance of tetravalent CYD vaccine was assessed in the repeat dose toxicity study and the biodistribution and shedding study in monkeys. Effects seen in the biodistribution and shedding study in monkeys were expected consequences of live attenuated viral inoculations and were limited to transient and minimal erythema reaction at the injection site that correlated with minimal to slight inflammatory reactions.

Paediatric use

Dengvaxia is proposed for paediatric use, no specific studies in juvenile animals were submitted. Assessment of safety in children will depend on clinical data.

Impurities

A number of vaccine impurities were toxicologically qualified.

Nonclinical summary and conclusions

Summary

- Studies in nonhuman primates (NHPs) with monovalent CYD-1, 2, 3 or 4 vaccine showed that each vaccine virus injected SC elicited a low, transient viraemia, and induced high titres of neutralising antibodies to homologous virus, with some cross-

[54] Category B2: Drugs which have been taken by only a limited number of pregnant women and women of childbearing age, without an increase in the frequency of malformation or other direct or indirect harmful effects on the human fetus having been observed. Studies in animals are inadequate or may be lacking, but available data show no evidence of an increased occurrence of fetal damage.
[55] Bartel A, et al. Breast milk as a possible route of vertical transmission of dengue virus? *Clinical Infectious Diseases* 57: 415-417 (2013).

reactivity to heterologous serotypes. Immunogenicity and viraemia of tetravalent CYD were consistent across Phase I, II and III lots. No viraemia was detected after a second vaccine dose. Neutralising antibody responses appeared durable for up to a year, the maximum period studied, in NHPs.

- Immunological responses to the YF 17D vector, and cell-mediated responses, were not investigated in nonclinical studies. In CYD vaccine the dengue NS1 and NS3 sequences which contain the main T-cell epitopes have been replaced with the corresponding YF segments.
- When combined into the tetravalent vaccine, neutralising antibody responses were mainly due to serotypes 1 and 4, and responses against serotypes 2 and 3 were low or absent, indicating interference. Serotype 4 viraemia was predominant. Interference between components of live attenuated vaccines has been reported for dengue and other live virus vaccines. The causes of interference probably involve serotype differences in replication and intrinsic immunogenicity.
- Studies in cynomolgus monkeys investigated various interference mitigation regimens, including sequential bivalent administration, decreasing the dose of the dominant serotypes, simultaneous bivalent injection into each arm, one or two month interval between doses, priming with YF 17D vaccine, and a third dose, one year after the first. Each of these regimens had a positive effect, but the three-dose regimen was the most successful.
- Dengue protection studies were conducted in NHPs. There is no recognised immune correlate of protection. A major limitation of the studies was the failure of NHPs to exhibit signs of dengue disease, hence protection was assessed in terms of diminution or absence of viraemia. An early study with monovalent CYD-2 vaccine at a SC dose of 2, 3, 4 or 5 $\log_{10}$ PFU showed full protection of all monkeys against viraemia induced by a SC challenge dose of 5 $\log_{10}$ PFU of wt DENV 63 days after immunisation.
- A SC challenge study (4 to 5 $\log_{10}$ $CCID_{50}$ of wt DENV-1, 2, 3 or 4) in cynomolgus monkeys 6 months after a single SC dose of tetravalent vaccine showed that 22/24 monkeys were fully protected (no viraemia), and 2/24 were partially protected. The two partially protected monkeys had low levels of neutralising antibodies against the challenge virus. All challenged monkeys showed a strong anamnestic antibody response. Three fully protected monkeys had neutralising antibody titres ($PRNT_{50}$) ≤ 20, suggesting contributions from cross-neutralising antibodies, and/or T-cell memory responses to protection.
- A high dose (7 $\log_{10}$ $CCID_{50}$) IV challenge with virulent wt DENV-2 in cynomolgus monkeys 8 months after a booster dose, 2 months after the first monovalent CYD-2 or tetravalent CYD dose, showed full protection (no viraemia) in 2/18 monkeys, and partial protection (viraemia reduced approximately 240-fold) in the rest. All monkeys showed a strong anamnestic neutralising antibody response to all 4 DENV serotypes post-challenge.
- Cross-protection in vitro was shown with sera from immunised monkeys against a panel of 82 wt dengue isolates, including the majority of existing genotypes, and all 4 serotypes, and pooled human sera against parental dengue and WHO reference dengue serotypes, and Asian and Latin America isolates.
- The low levels of protection of CYD vaccine against DENV-2 in clinical trial CYD23 led to nonclinical studies of potential causes. In vitro data generated in monocytic and dendritic cell models showed CYD-2 virus was less infectious than the other 3 serotypes and similar observations were also made in NHPs. Low viraemia, and low immunogenicity coupled with possible interference of other serotypes may have an impact on CYD-2's ability to elicit strong protective immunity.

- In post CYD23 in vitro studies, seroneutralisation assays in Vero cells using CYD23 subjects' sera indicated responses induced by tetravalent CYD vaccine were capable of neutralising all parental and wt DENV-2 strains (n = 7) circulating in Thailand at the time of the clinical trial. However, neutralising antibodies have been previously reported not to be accurate correlates for protection in vivo. Similarly no in vitro antibody enhancement of DENV-2 activity was observed in FcγRIIa+CV1 cells, however a more appropriate model containing both FcγRIIa and FCγRIIb as present in dengue target cells would have provided more conclusive results. The potential for disease enhancement is best assessed by clinical data.
- In humans, a second dengue infection with a different serotype to the primary infection is associated with a higher risk of severe disease, hence there is a theoretical risk of sensitisation due to CYD vaccination. NHPs do not develop dengue disease after SC inoculation, hence sensitisation was investigated in terms of enhancement of viraemia. No enhanced viraemia was observed in CYD vaccinated cynomolgus monkeys challenged with wt dengue, or in monkeys with pre-existing flavivirus immunity due to YF vaccination, or with sequential heterologous bivalent CYD vaccination. However, enhancement of viraemia due to secondary heterologous wt dengue infection, which has been reported only for serotype 2 in rhesus monkeys, has not been observed in cynomolgus monkeys, hence the validity of the model is uncertain.
- No nonclinical data were submitted on cross-reactive antibodies to Zika virus, or other flaviviruses such as Murray Valley encephalitis or Kunjin viruses, which also raise a theoretical risk of disease enhancement by CYD vaccine.
- The YF 17D vaccine virus has been associated with rare cases of neurotropism and viscerotropism in humans. Tetravalent CYD vaccine was tested for neurovirulence in cynomolgus monkeys by direct intracerebral injection of the human dose according to WHO procedures for YF vaccine, using YF 17D as a comparator. CYD vaccine had significantly lower neurovirulence overall. Viraemia was transient and below the WHO requirements for YF 17D vaccines.
- A GLP-complaint biodistribution and toxicity study in cynomolgus monkeys administered the human dose of CYD vaccine SC showed that virus distribution was transient and limited to the injection site, lymphoid tissues and/or liver. No viral RNA was detected in nervous tissue. On day 22 post-dose, virus was only detected in the injection site tissues and draining lymph nodes in a few monkeys. There was no virus shedding in body fluids. Toxicological findings were limited to a transient minimal to slight inflammatory reaction at the injection site.
- The YF 17D virus is highly attenuated and has a long history of safety in humans. However, '*… the virulence of live recombinant vaccines cannot be predicted from that of the viral vector, even when the vector by itself is already attenuated for humans …'* (EMA guideline).[56] Vaccine virus attenuation was shown by the lack of adverse findings in the NHP toxicity and biodistribution studies, lower neurovirulence than YF 17D vaccine in cynomolgus monkeys, and low levels of transient viraemia in all studies in NHPs (similar to YF vaccine, and lower than the parent dengue viruses).
- Studies of genetic stability in vitro and in NHPs in vivo indicated low potential for reversion for virulence, either by back mutations, or by recombination with flaviviruses. Chimerisation also contributes to attenuation, as CYD vaccine remained highly attenuated in monkeys despite replacement of the entire YF 17D backbone with the corresponding virulent Asibi parent sequence.

[56] EMA CHMP (2010) Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (EMA/CHMP/VWP/141697/2009).

- A GLP-compliant toxicity study conducted with three consecutive human SC doses of tetravalent CYD vaccine in cynomolgus monkeys showed no adverse effects, including injection site reactions. All monkeys had an antibody response to all 4 serotypes.
- No genotoxicity or carcinogenicity studies were performed. This was consistent with nonclinical vaccine guidelines.
- Reproductive toxicity was studied in mice and rabbits, but not in NHPs. Antibody responses were limited in mice, and viraemia was very low and limited to the day after injection in rabbits. Mice given a single IV dose (5, 6.5, 8 $\log_{10}$ $CCID_{50}$) of tetravalent CYD on GD 6, 9 or 12 showed an increase in post-implantation loss, reduced fetal bodyweights, and reduced ossification, associated with maternotoxicity. There was no virus transfer to fetuses, although it occurred in a dose-ranging study. A lactation toxicity study in mice given a single IV dose on lactation Day 14 showed maternal toxicity, but no effects on pups. No virus was detected in pups or milk.
- A combined reproductive and developmental toxicity study in female rabbits given the human dose of tetravalent CYD IV twice before mating and three times during gestation showed no adverse effects. Anti-CYD antibodies against all 4 serotypes were detected in the sera of all dams, with transfer to fetuses and pups.
- CYD vaccine is contraindicated in pregnancy, as for other live attenuated virus vaccines. The advice to avoid pregnancy for 4 weeks after any dose of CYD vaccine is consistent with nonclinical biodistribution and viraemia data. In view of the limitations of the mouse and rabbit models, a pregnancy category of B2 is recommended.

Conclusions and recommendation

- The nonclinical data in support of Dengvaxia consisted of immunogenicity, protective efficacy, safety pharmacology (neurovirulence), biodistribution, and repeat dose toxicity studies in non-human primates (NHPs), and reproductive and developmental toxicity studies in mice and rabbits. All safety studies were GLP compliant.
- CYD vaccine induced neutralising antibodies against all 4 serotypes in NHPs, the antibody responses appeared durable for up to a year, the maximum period studied. However, comparison of monovalent and tetravalent vaccine indicated interference between serotypes. Interference between components of live attenuated vaccines has been reported for dengue and other live virus vaccines. A 3 dose regimen was the most successful in terms of balancing neutralising antibody responses to all 4 serotypes.
- Immunological responses to the YF 17D vector, and cell-mediated responses, were not investigated in nonclinical studies, although recommended by the EMA guideline.[57] A theoretical limitation of CYD vaccine is that the DENV NS1 and NS3 sequences which contain the main T-cell epitopes have been replaced with the corresponding YF sequences.
- Dengue protection studies were conducted in NHPs. A major limitation of the studies was the failure of NHPs to develop dengue disease; hence protection was assessed in terms of reduction or absence of viraemia. Most (22/24) vaccinated NHPs were fully protected (no viraemia) against a moderate SC challenge, and partially protected against a high dose IV challenge. Several monkeys were protected despite low neutralising antibody titres, possibly due to cell-mediated immunity. In the absence of a recognised immune correlate of protection, evaluation of protection will also depend on clinical efficacy data.

[57] EMA (2010). Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (EMA/CHMP/VWP/141697/2009).

- Cross protection in vitro was shown with monkey and human sera against representative panels of wt DENV.
- In humans, a second dengue infection with a different serotype to the primary infection is associated with a higher risk of severe disease; hence there is a theoretical risk of sensitisation due to CYD vaccination. NHPs do not develop dengue disease after SC inoculation, hence sensitisation was investigated in terms of enhancement of viraemia. No enhanced viraemia was observed in any of the NHP studies. However, the validity of the model is uncertain, as viraemia enhancement has not been reported in cynomolgus monkeys with 2^0 heterologous wt infection.
- No nonclinical data were submitted on cross-reactive antibodies to Zika virus, or other flaviviruses such as Murray Valley encephalitis or Kunjin, which also raise a theoretical risk of sensitisation by CYD vaccine. The potential for sensitisation will need to be fully addressed in clinical studies over the longer term.
- Nonclinical biodistribution, toxicity and neurovirulence studies in NHPs showed that the vaccine viruses are highly attenuated, with a transient minimal to slight inflammatory reaction at the injection site being the only finding. Viraemia was low and transient, no virus shedding was detected. Genetic stability studies indicated that reversion to virulence by back mutation or recombination is very unlikely.
- Reproductive toxicity studies in mice and rabbits did not show any direct adverse effects on the fetus, however due to limitations of the models a pregnancy category of B2 is recommended. CYD vaccine is contraindicated in pregnancy, as are other live attenuated vaccines. Prevention of pregnancy for one month after any dose is consistent with biodistribution and viraemia data.
- There are no nonclinical objections to registration, provided that efficacy and the potential for sensitisation to flavivirus disease have been satisfactorily addressed in clinical studies.
- Registration will also be subject to evaluation by the Office of the Gene Technology Regulator.

V. Clinical findings

A summary of the clinical findings is presented in this section. Further details of these clinical findings can be found in Attachment 2.

Introduction

Clinical rationale

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

Guidance

The CDP of the CYD dengue vaccine was initiated in 2002 in accordance with guidance from the EMA and WHO.

Contents of the clinical dossier

A total of 28,894 subjects aged 9 months to 60 years were randomised in the studies presented in the Application, to receive at least one injection of a tetravalent CYD dengue vaccine, regardless of the formulation. Among these subjects:

- 28,653 received at least one injection of the CYD dengue vaccine (final formulation regardless of the schedule) and were included in the safety database, in which the occurrence of serious adverse events (SAEs) and adverse events of special interest (AESIs) was assessed. A total of 27,643 subjects received the final formulation with the final schedule.
- 7,576 subjects provided data to assess the reactogenicity of the final formulation of the CYD dengue vaccine.

The main objectives of the CDP were to characterise the candidate vaccine in terms of efficacy, safety and immunogenicity profiles, when assessed in different regions, in different age groups and in populations with various degrees of endemicity, from highly endemic to non-endemic. There is currently no licensed dengue vaccine and no immunological correlate of protection has currently been established. Therefore, the efficacy of the CYD dengue vaccine has been assessed in endemic areas in one proof of concept (PoC) Phase IIb mono-centre Study CYD23 conducted in Thailand, and 2 pivotal Phase III studies performed in 10 countries of southeast Asia Pacific (AP) and Latin America (Lactam) (Study CYD14 in AP and Study CYD15 in Lactam).

The majority of studies, including the studies assessing the final formulation of the vaccine given with the final schedule, were randomised, controlled and at least blind-observer studies. All serology testing was performed in a blinded manner. During the long-term safety follow-up of the efficacy studies, investigators and subjects remain blinded to the vaccine received during the Active Phase of the study, that is, from inclusion to 25 months after the first injection.

As of December 2015, the CDP includes 25 clinical studies, completed (21) or on-going (4):

- 5 Phase I studies
- 14 Phase II studies
- 6 Phase III studies

A total of more than 41,000 subjects have been enrolled in clinical studies including more than 28,500 subjects from 9 months through 60 years of age exposed to at least one injection of the final tetravalent CYD dengue vaccine formulation, regardless of the administration schedule. Among these subjects, 21,215 subjects were aged 9 through 60 years and received at least one injection of the final formulation of the CYD dengue vaccine, regardless of the schedule. Results from 24 clinical studies are described in the present application (Study CYD56 is ongoing).

Phase I and early phase II studies

The first clinical study conducted as part of the CDP of the vaccine, Study CYD01, assessed safety and immunogenicity of a monovalent vaccine against dengue virus serotype 2. All remaining Phase I studies (Studies CYD02, CYD04, CYD05 and CYD06) evaluated the safety of the CYD dengue vaccine first in adults from non-endemic areas (Studies CYD02 and CYD04) and in a second step in adults and children in non-endemic (CYD06) and in an endemic area (Study CYD05). The studies were first conducted in non-endemic areas to collect data from subjects who are non-immune to flaviviruses, and especially to dengue. Because of the risk of severe disease, it was important that subjects included in the first studies not be at risk of natural infection with dengue or other flaviviruses. These Phase I studies together with 3 Phase II studies (Studies CYD10, CYD11 and CYD12) provided data on safety and immune response induced by several formulations of the vaccine and

different schedules of vaccination. The results of these 8 studies supported the selection of the final vaccine formulation and schedule, that is, approximately 5 log10 $CCID_{50}$ of each live, attenuated, dengue serotype 1, 2, 3, 4 virus given as 3 injections 6 months apart.

Late phase II studies

Based on safety and immunogenicity results from the above-mentioned studies, 5 additional Phase II studies (Studies CYD13, CYD22, CYD24, CYD28 and CYD30) were initiated in different endemic countries in AP and Lactam to further evaluate the safety and immunogenicity of the CYD dengue vaccine in different populations (that is, age, baseline flavivirus (FV) status, region) following 3 injections of the final formulation administered 6 months apart. A PoC efficacy study (Phase IIb) was then initiated in Thailand (Study CYD23) in children aged 4 to 11 years, for whom a safety follow-up is ongoing (Study CYD57). An additional Phase II study was initiated in India (Study CYD47) to assess safety and immunogenicity of the CYD dengue vaccine in Indian subjects, as required by local Authorities for registration.

A PoC co-administration Phase II study (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.

Additionally, clinical investigations into a shorter schedule adapted to traveller/non-endemic populations were initiated via 2 Phase II studies in adults in the US (Studies CYD51 and CYD56). Study CYD51 is completed and Study CYD56 is ongoing.

Phase III studies

Two Phase III efficacy studies, each statistically powered to independently demonstrate efficacy, were designed and prepared to be carried out in parallel in 10 endemic countries: Study CYD14 (5 countries in AP, 2 to 14 year old children) and Study CYD15 (5 countries in Latin America, 9 to 16 year old children and adolescents). Enrolment started prior to availability of the supportive PoC Phase IIb results.

Four other Phase III clinical studies (Studies CYD17, CYD29, CYD32 and CYD33) were also conducted:

- Study CYD17 had a primary objective to demonstrate the consistency of 3 commercial scale lots (Phase III lots) in a non-endemic population. Study CYD17 also provided safety and immunogenicity information on the new bulk process of Phase III lots in comparison to Phase II lots and data bridging the Phase II lots to the Phase III lots.
- Study CYD32 was a Phase III study to evaluate safety and immunogenicity of the vaccine in a paediatric population in Malaysia.
- The 2 other Phase III studies investigated concomitant administration with vaccines given to infants and toddlers below 2 years of age: YF co-administration with the first injection of CYD dengue vaccine from 12 months of age in Peru and Colombia (Study CYD29) and co- administration of Detach-IPV booster with the second injection of the CYD dengue vaccine from 15 months of age in Mexico (Study CYD33).

Duration of follow-up

In all studies except Studies CYD04 and CYD06, safety was followed up to at least 6 months after the last injection.

The majority of the clinical database consists of subjects aged 9 months to 45 years at high risk of dengue disease. Less data are currently available in adults more than 45 years old: a total of 241 adults from 46 to 60 years of age received the CYD dengue vaccine in Study CYD17, conducted in a non-endemic area. No data are available from adult subjects aged 46 to 60 years living in an endemic area. In addition, a limited number of adults (668 subjects from 18 to 45 years old) in endemic population received the CYD dengue vaccine.

The clinical studies did not include adults aged more than 60 years old or children below 9 months. The safety database in subjects aged 9 through 60 years allows for the detection of very common, common and uncommon adverse events (AEs) as recommended by WHO[5].Thus, a possibility of rare (that is, with the frequency less than 0.1%) AEs going undetected cannot be excluded.

The clinical module includes reports for:

- 8 clinical pharmacology studies providing for PD/dosage and safety data.
- 10 Phase II efficacy and safety studies.
- Interim report on long term follow up Study CYD 57.
- One proof of concept study (Study CYD23).
- 2 pivotal efficacy/safety studies.
- 1 lot to lot consistency study.
- Case report forms with safety data.
- Safety integrated analysis report, immunogenicity and efficacy integrated analysis report.
- Immune and vero assay details.
- Literature references.

Paediatric data

Most of the studies submitted in this application include paediatric data.

Good clinical practice

All clinical studies evaluating the CYD dengue vaccine comply with the Quality Standards of the International Conference on Harmonisation (ICH) guidelines, the Food and Drug Administration (FDA) guidelines for Good Clinical Practice (GCP), EU Directive 2001/20/EC and the EMA guidelines on clinical evaluation of new vaccines clinical study reports in the submission state the studies complied with CPMP/ICH/135/95: Note for Guidance on Good Clinical Practice.

Pharmacokinetics

Not applicable.

Pharmacodynamics

In accordance with the EMA 'Guideline on Clinical Evaluation of New Vaccines';[58] the pharmacodynamic profile for the CYD dengue vaccine was defined by its immunogenicity profile.

[58] European Medicines Agency. Guideline on clinical evaluation of new vaccines. 19 Dec 2006 (EMEA/CHMP/VWP/164653/2005).

Dosage selection for the pivotal studies

Pharmacokinetics and pharmacodynamics: dose finding studies

The choice of parental strains, that is, the strains from which the vaccine was derived, within the assay was made in order to ensure optimal assessment of vaccine induced immune response by using the matched vaccine antigens.

The objectives of the first clinical studies were to define the formulation, the dosage and the schedule of administration of the CYD dengue vaccine. Eight clinical studies (5 Phase I studies and 3 Phase II studies) were conducted for that purpose. These studies were mainly conducted in adult subjects (except 2 Phase I studies conducted in children, adolescents and adults) living in non-endemic areas. Only 1 study, Study CYD05, was conducted in endemic areas (the Philippines).

Choice of formulation (tetravalent vaccine) and concentration

Tetravalent vaccine

Study CYD01 assessed safety and immunogenicity of a single dose of monovalent chimeric dengue 2 vaccine (Chimerivax-DEN2) containing 5 or 3 $\log_{10}$ plaque forming units (PFU)) and showed that one dose of monovalent chimeric dengue 2 vaccine induced a satisfactory immune response against serotype 2 and low seropositive rates to the other 3 serotypes (in YF non-immune subjects), confirming the need for a tetravalent vaccine.

A tetravalent vaccine against the 4 serotypes was tested in CYD04 and showed satisfactory safety and immunogenicity profiles in FV non-immune adults, and, in CYD05 and CYD06, in different age groups (2 to 45 years) and FV backgrounds. The immunogenicity response varied across populations due to co-factors (that is, age, baseline status, region). Seropositive rates against all 4 serotypes after 3 injections of a tetravalent formulation ranged from 39.1% (Study CYD04, FV non- immune adults) to 85.0% (Study CYD05, FV immune adults, adolescents, and children).

The choice of a tetravalent formulation was confirmed by the use of sequential or simultaneous bivalent formulations in Study CYD11, which did not improve the immune response compared to the tetravalent formulation.

5555 formulation

The Phase I Study CYD01 showed that single administration of ChimeriVax-DEN2 at either 5 $\log_{10}$ PFU or 3 $\log_{10}$ PFU was safe and immunogenic in both YF non-immune and YF immune subjects. Based on these findings, the mid-range concentration of 4 $\log_{10}$ $CCID_{50}$ of tetravalent ChimeriVax DEN was chosen for Study CYD02. The concentration of 5 $\log_{10}$ $CCID_{50}$ of tetravalent ChimeriVax DEN was chosen for Studies CYD04, CYD05, and CYD06 and provided an immune response against all four serotypes. In Study CYD12, the safety and immunogenicity of 3 CYD dengue vaccine formulations were assessed: 5555 (5 $\log_{10}$ for each of the 4 serotypes), 5553 (5 $\log_{10}$ for serotypes 1, 2, and 3 and 3 $\log_{10}$for serotype 4), and 4444 (4 $\log_{10}$ for each of the 4 serotypes). The 5553 formulation was intended to improve the immune response by taking into account the immuno dominance of serotype 4 observed in previous studies.

In Study CYD12, the 5555 formulation showed a trend toward higher seropositivity rates to the 4 serotypes after the third injection (62.9%) than the 5553 formulation (59.3%) and the 4444 formulation (53.3%). The 4444 formulation induced similar GMTs to those with the 5555 formulation, however, seropositivity rates to all 4 serotypes tended to be lower. Considering the GMTs, the 5553 formulation elicited the highest GMTs to serotypes 1, 2, and 3 (57.1 (1/dil) to 114 (1/dil)), while the GMT for serotype 4 (25.9 (1/dil)) was lower than that with the other formulations. The 4444 formulation elicited similar GMTs to the 5555 formulation but with a trend toward lower GMTs for serotype 3. The different

vaccine formulations assessed in Study CYD12 showed that different concentrations of a given serotype can impact the immune response to the other serotypes. Safety profile of the different formulations was acceptable and similar. The formulation at around 5 log_{10} $CCID_{50}$ per serotype (5555) reliably provided an immune response against all 4 serotypes after 3 injections in various populations, regardless of age, region, FV status at Baseline, and was selected for further Phase II and Phase III studies.

Choice of vaccination schedule

The vaccination schedule was selected mainly based on the results from Phase I studies, that is, Studies CYD02, CYD04, CYD05 and CYD06. The choice of schedule was then supported by data from Phase II studies, particularly Study CYD12. The main parameters for the selection of the number of injections and the dosing interval were the achievement of an acceptable immune response against the 4 dengue serotypes in a timely manner, in all subjects included in the CDP, that is, regardless of age, region and of baseline immune status to dengue.

In Studies CYD04, CYD05 and CYD06, groups having received the tetravalent vaccine administered at a 3-injection regimen at 0, 3/4, 12 months provided information on different categories (adults, adolescents, and children), different regions (non-endemic USA and Latin America, and endemic Asia Pacific) and different baseline FV status at baseline (FV non-immune versus immune subjects). The data showed that increasing the interval between injections had a beneficial effect on the overall immunogenicity outcome.

Overall across the studies, there was a trend toward progressive increase in GMTs and seropositivity rates after each dengue injection, with a decrease between injections. After 3 injections of the 5 log_{10} concentration, the seropositivity rates against all 4 serotypes ranged from 39.1% (Study CYD04, FV non-immune adults) to 85.0% (Study CYD05, FV immune adults, adolescents, and children). A predominant response to serotype 4 was observed after the first injection of the CYD dengue vaccine in these 3 studies. The second and the third injections induced an increase in GMTs for all serotypes in baseline FV non-immune subjects.

Within Studies CYD05 and CYD06 including children, adolescents, and adults in both non-endemic and endemic regions, younger children appear to benefit the most from the third injection to achieve a broad immune response against the 4 serotypes. In addition, the benefit of the third injection in terms of immune response was more marked in baseline FV non-immune populations, who tended to have a lower immune response after 2 injections in Studies CYD04, CYD06, and within CYD05 conducted in an endemic region.

Three injections provided the best approach to achieve a consistent immune response against all 4 serotypes in all evaluated populations.

The interval between the first and second injections was evaluated in Phase I Studies CYD04 and CYD05 which investigated 3 injections of the approximately 5 log_{10} concentration at 0, 3/4 and 12 months in the dengue group and 2 injections at 0 and 8/9 months in the Control group.

An intra-study group comparison between Study CYD05 subjects who received 2 injections of the CYD dengue vaccine either 3/4 months apart or 8/9 months apart showed a trend toward higher GMTs when the second injection was given 8 to 9 months after the first injection in children and adolescents in endemic regions (Study CYD05). These observations suggested that increasing the interval between injections contributes to a higher immune response. Study CYD12 was the first Phase II study testing the final 5 log_{10} concentration with a 6-month interval between injections. Study CYD12 immunogenicity results confirmed that 3 injections at a 6-month interval provided a satisfactory immune response to the 4 serotypes, with 62.9% of FV non-immune adult subject being seropositive against all 4 serotypes. Additionally, Phase I and II study

observations suggested that increasing the interval between injections resulted in an increase in Ab response.

Cellular immune response

The CMI response was assessed in a subset of adult and adolescents subjects in Studies CYD04, CYD10, CYD11, and CYD28. First, there was no evidence of increase in inflammatory responses after immunisation with the CYD dengue vaccine; no increase of innate pro-inflammatory cytokine production or other markers of sensitisation to severe outcomes of dengue were observed. Second, regarding adaptive T cell responses, in volunteers seronegative at baseline, the CYD dengue vaccine induced serotype-specific Th1/Tc1 responses to structural antigens from all four dengue serotypes, as well as CD8/Tc1 responses to YF17D non-structural (NS) 3 antigen. After three injections of CYD dengue vaccine, a balanced cellular response was induced against all four serotypes, and these serotype-specific T cell responses paralleled the neutralising Ab responses measured by PRNT50 assay. CD8/Tc1 responses directed against dengue NS3 were also boosted by the CYD dengue vaccine in individuals dengue-immune at baseline. Regarding the cytokine profile, the vaccine induced a cellular response with a Th1/Tc1 profile wherein interferon-γ (IFN- γ) dominates over tumour necrosis factor α (TNF-α) and Th2 cytokines including interleukin-13 (IL-13). This suggests that over-inflammatory and potentially detrimental dengue-specific responses would not be recalled upon a subsequent dengue infection in vaccinees, while dengue- specific T cell help to B cells would be beneficial to increase and accelerate neutralising Ab responses.

Evaluator's conclusions on dose finding for the pivotal studies

There were a number of Phase I and II studies performed to examine the dose and scheduling of the CYD vaccine. These were performed in adults, and then children, both in non-endemic and endemic areas. They were conducted initially in non-endemic regions to ensure that there were no negative consequences from subsequent natural infection. The benefit of 3 injections on the seropositivity rate was observed, especially in younger age groups and/or baseline FV non-immune populations. There were no substantial differences in terms of GMTs levels post-injection 3 compared to post-injection 2, but as GMTs decreased between injections, subjects benefit from the third injection to increase the levels of GMTs.

The schedule with 3 injections at 6 month intervals was further confirmed with subsequent studies conducted in different settings (age, baseline FV status) using the same Phase II lots of the final formulation with the chosen schedule.

Efficacy

Studies providing efficacy data

The evaluation of efficacy of the CYD dengue vaccine is based on the vaccine efficacy (VE) observed in the 2 pivotal Phase III efficacy Studies CYD14 and CYD15. Supportive clinical data were obtained from CYD23, a Phase IIb PoC efficacy study. Additionally, immunogenicity data were obtained from 16 studies in subjects aged 9 months to 60 years that assessed the final formulation of the CYD dengue vaccine given in a 3 dose- schedule at 6 month intervals: Studies CYD12a, CYD17 and CYD51 in non-endemic regions and Studies CYD08, CYD13, CYD14, CYD15, CYD22, CYD23, CYD24, CYD28, CYD29, CYD30, CYD32, CYD33 and CYD47 in endemic regions. Specific analyses of immune responses as they related to efficacy were performed in data generated in efficacy Studies CYD14 and CYD15.

The main objective of the immunogenicity studies was to describe the humoral immune response induced by 3 injections of CYD dengue vaccine administered 6 months apart in both endemic and non-endemic populations with age, previous exposure to dengue and other FV and region as key variables.

The main objective of the efficacy studies was to demonstrate VE of the CYD dengue vaccine in preventing virologically confirmed dengue (VCD) cases, in accordance with WHO guidelines on dengue vaccine evaluation. The large scale efficacy studies also allowed for the assessment of the relationship between the occurrence of VCD cases and the level of neutralising Ab titre 28 days after the third injection.

Criteria for assessment

Humoral immune response

The PRNT assay was used to assess the immunogenicity of the CYD dengue vaccine through the measurement of neutralising Ab at varying time points. The PRNT was performed by the applicant's laboratory, for all studies that assessed the final formulation.

Immunological and virological assay methods

For the majority of subjects, the humoral immune response was assessed before and approximately 30 days after each injection.

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

- GMTs expressed in reciprocal of dilution (1/dil) for each serotype.
- GMTRs from baseline to post-vaccination for each serotype.
- Seropositivity rate, defined as the proportion of subjects with a neutralising 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. Initial assessment of immune response to dengue vaccine was based upon experiences with JE vaccination and the associated correlate of protection primarily in non-endemic populations.[8] As experience accumulated in endemic populations of different ages and regions, GMT became the most important criteria for the dose assessment.

Cellular immune response

In order to further characterise the immune response induced by the CYD dengue vaccine and as recommended in the WHO and EMA guidelines, cell-mediated immunity was also assessed in some studies in adolescents and adults in endemic and non-endemic regions (Studies CYD04, CYD10, CYD11 and CYD28).

Pivotal or main efficacy studies

The two large scale Phase III efficacy studies were randomised, placebo controlled, observer blinded and stratified by age. Studies CYD14 and CYD15 are described together because of the identical structure. These trials were similar in all respects apart from geographical location. Each individual study was sufficiently powered to demonstrate significant efficacy of the CYD dengue vaccine in preventing the occurrence of VCD due to any serotype after 3 injections, given 6 months apart with a time window of ± 20 days for the second and third injections. The choice of the countries and sites for the Phase III efficacy studies was based on national surveillance data and available data from epidemiological studies showing that these countries were highly endemic and had evidence of all 4 serotypes circulating. The choice was confirmed by the results of the 2 prospective cohort studies conducted by the applicant prior to the initiation of the studies.

These data provided an estimate of the dengue attack rate in the study target population (3.4% of VCD cases in Asia Pacific and 1.2% of VCD in Latin America).

Evaluator's conclusions on efficacy

Efficacy was consistently demonstrated in the pivotal Phase III efficacy studies conducted over a 2-year period in two distinct geographic regions with circulation of the 4 serotypes in both. Overall, 55% to 65% VE was observed in preventing occurrence of VCD cases due to any serotype after at least one injection of the CYD dengue vaccine. Significant VE was also observed in preventing the occurrence VCD cases due to each serotype after at least one injection of the CYD dengue vaccine. This varied according to the serotype: moderate efficacy was observed for serotypes 1 and 2 and high efficacy was observed for serotypes 3 and 4.

A number of factors influence the overall VE of the CYD dengue vaccine. At the study level, the distribution of serotypes in the region or country influenced overall efficacy outcomes: when serotype 2 predominated, overall efficacy was lower. At an individual level, the subjects' age at vaccination, baseline immune status, and level of the response to the vaccine all had an effect on efficacy outcomes. Age also seems to influence the VE, with increasing VE in older subjects.

The primary endpoint of the two pivotal studies however was the prevention of confirmed dengue fever cases. This was shown in both studies in all age groups, in all countries and for all serotypes (although the vaccine is least effective for serotype 2). It was also more effective for older children. As a group, subjects aged 9 to 17 years showed a more favourable profile than the lower age groups. Subjects aged 2 to 5 years showed the lowest Ab responses to the vaccine and as a consequence, lower efficacy.

These pivotal studies had immunogenicity subsets which showed the development of antibodies, increasing levels with increasing age and prior exposure (baseline antibodies). The dengue immune status at baseline is an important factor influencing the response to the vaccine. Dengue immune subjects at baseline had higher post-injection Ab responses than age matched dengue non-immune subjects. Since subjects aged 2 to 5 years show a much higher proportion of dengue non-immune subjects at baseline, as expected, they had lower Ab responses and therefore lower efficacy. Subjects from endemic areas in the claimed population had the highest GMT while subjects in the 9 to 15 months old group had the lowest. Adults from endemic regions had overall higher GMTs than children and adolescents evaluated from the 2 pivotal efficacy studies.

These findings suggest that age is a key factor for predicting baseline status, reflecting increased accumulative exposure to dengue infection over time, subsequently influencing vaccine elicited immune response and thereby impacting efficacy outcomes.

Long-term efficacy

Currently, no data on the long-term efficacy of the vaccine is available in the intended population for use. Therefore, no conclusion can be drawn on long-term vaccine efficacy over time against all symptomatic VCD cases in the claimed population.

In order to further assess long-term efficacy, additional data on efficacy endpoints against all symptomatic VCD cases are now being captured in the long-term follow-up of the Phase III efficacy studies. In addition, booster will be evaluated in follow-up studies from Phase II Studies CYD63 and CYD64. For Study CYD63, a subset of subjects included in Study CYD28 (Phase II study conducted in Singapore) and who were 9 to 45 years at the time of inclusion will be asked to participate to Study CYD63 to receive a booster injection around 5 years after the last injection received in Study CYD28. For Study CYD64, a subset of subjects included in Study CYD13 (Phase II study conducted in Mexico, Honduras, Colombia and Puerto Rico) and in Study CYD30 (Phase II study conducted in Brazil) and

who were 9 to 16 years at inclusion in these studies, will be asked to participate to Study CYD64 to receive a booster injection 4 to 5 years after the last injection received in Studies CYD13 or CYD30. These studies will assess the safety and immunogenicity of boosting.

Safety

Studies providing safety data

Both Studies CYD14 and CYD15 provided safety data.

Overall, regardless of age, 21 clinical studies that used CYD dengue vaccine containing approximately 5 $\log_{10}$ $CCID_{50}$ per serotype are included in the integrated safety analysis. A total of 16 studies administered CYD dengue vaccine in the final immunisation schedule of 3 injections administered 6 months apart and were considered the main studies for the integrated safety.

5 studies administered CYD dengue vaccine in other immunisation schedule and were considered secondary studies providing supportive safety data.

Criteria for analysis

Pre-defined solicited reactions (up to 14 days); local and systemic, 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 Studies 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 with the final schedule.

While all SAEs are collected in the efficacy Studies CYD14 and CYD15 up to 5 years post-injection 3, a limited set of SAEs (including related SAEs and hospitalised dengue cases) are collected in Studies CYD05, CYD22, CYD57 and CYD28 during the long-term follow-up of safety.

AESIs have been defined for the CYD dengue vaccine in all studies and are 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. Vaccine viraemia was evaluated as an indicator of safety and is defined as the presence of vaccine viruses in the blood. Vaccine viraemia was also assessed in subjects with acute febrile episodes within 28 days after vaccine injection, in dengue endemic regions (in Studies CYD08, CYD13, CYD22, CYD24, CYD28, CYD30, CYD33 and CYD23), to determine whether the fever was linked to the vaccine (positive vaccine viraemia) or to dengue infection, in accordance with WHO guidelines. Dengue cases were also collected during the clinical development of the CYD dengue vaccine as an assessment of the safety of the vaccine and in accordance with WHO guidelines.[59]

[59] World Health Organization. Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). WHO Expert Committee on Biological Standardization. WHO Technical Report Series No. 979, 2013. Annex 2; World Health Organization. Guidelines for the clinical evaluation of dengue vaccines in endemic areas. Department of Immunisation, Vaccines and Biologicals. Geneva, WHO, 2008 (WHO/IVB/08.12).

Severe dengue

Any of the serotypes can cause severe dengue and fatal disease. The occurrence of severe virologically confirmed dengue (SVCD) cases was assessed throughout the Active Phase of the Phase III efficacy studies and during the long-term safety follow-up.

Patient exposure

The safety database for the CYD dengue vaccine consists of all subjects who received at least one injection of the tetravalent CYD dengue vaccine containing approximately 5 $\log_{10}$ $CCID_{50}$ of each serotype administered with the final vaccination schedule, that is, 3 injections 6 months apart. Data were pooled and presented by age of study subjects: including adults (18 to 60 years), adolescents (12 to 17 years), children (2 to 11 years, further divided in 2 to 5 and 6 to 11 years), and infants and toddlers (below 2 years of age). To date, a total of approximately 28,900 subjects aged 9 months to 60 years have 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 received at least one injection of the final formulation, regardless of the schedule. Among these subjects, 21,215 subjects were in the claimed age indication (9 through 60 years of age). Approximately 20,600 subjects aged 9 to 60 years received at least one injection with the final schedule and approximately 19,700 subjects aged 9 to 60 years received 3 injections of CYD dengue vaccine with the final schedule. This database should allow for the detection of very common, common and uncommon AEs in accordance with WHO guidelines.

Safety issues with the potential for major regulatory impact

No safety concerns were raised from the analysis of the biological safety parameters. The majority of subjects had biological values within normal ranges both at baseline and after any CYD dengue vaccine injection and at any day. The most frequent abnormalities after any CYD dengue vaccine injection and any day were decreased haematocrit and decreased haemoglobin, decreased and increased WBC count, and increased AST.

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 neutropaenia.

Immunogenicity and immunological events

Study CYD14 AESIs

No immediate anaphylactic shock has been reported. The proportions of subjects who experienced at least 1 non-serious potential allergic reaction within 7 days after any injection was low, ranging from 0.5% in adolescents to 1.2 % in adults, and comparable in the Dengue Group and in the Placebo Group. The proportions of subjects in the claimed indication who experienced at least 1 non-serious potential allergic reaction within 7 days after any injection was 1.2% in the Dengue Group and 0.3% in the Placebo Group in adults and 0.5% in both groups in subjects aged 9 to 17 years. Less than 0.1% of subjects experienced at least 1 serious potential allergic reaction in the Dengue Group in subjects aged 9 to 17 years and adults. Only 5 subjects experienced a serious potential allergic reaction in the Dengue Group (1 adult and 4 subjects aged 9 to 17 years): 4 subjects experienced asthma or asthmatic crisis and had a relevant medical history of asthma, asthmatic bronchitis, or bronchial obstructive symptoms; 1 subject experienced urticaria and had a history of allergic rhinitis. Two of these serious potential allergic reactions (urticaria and asthma) were assessed as related to the study vaccine.

No confirmed viscerotropic and neurotropic events were reported in any study. There was no excess of clinically severe VCD cases in vaccine recipients compared to controls in the observation period, mainly 25 month of follow-up after first dose.

Study CYD15 AESIs

Non-serious hypersensitivity/allergic reactions within 7 days after any injection

Seven subjects experienced at least one non-serious hypersensitivity/allergic reaction AESI within 7 days of any injection i.e., 4 in the CYD Dengue Vaccine Group (2 subjects experienced dyspnoea, 1 subject developed generalised erythema, and 1subject experienced asthmatic crisis and urticaria) and 3 in the Control Group (asthma (2 episodes), and pruritus). Most were of Grade 1 intensity except asthmatic crisis and urticaria that were of Grade 3. They did not lead to discontinuation from further injections except asthmatic crisis and urticaria. In addition to asthmatic crisis and urticaria, one of the 2 episodes of dyspnoea, generalised erythema in the CYD Dengue Vaccine Group, and pruritus in the Control Group were assessed as related to study product.

Serious hypersensitivity/allergic reactions within 7 days after any injection

Five subjects experienced at least one serious AESI hypersensitivity/allergic reaction within 7 days of any injection: 4 in the CYD Dengue Vaccine Group (2 subjects had asthma, 1 subject had asthmatic crisis and 1 subject developed urticaria) and 1 in the Control Group (asthma). In the CYD Dengue Vaccine Group, one case of asthma and the case of urticaria occurred within 24 hours of the vaccine injection (post-Injection 1 and post-Injection 2, respectively). These 2 SAEs did not require hospitalisation but subjects were discontinued from the following injections. Both SAEs were assessed as related to vaccination by both the Investigator and the Sponsor. All serious AESIs resolved.

Serious skin reactions

Discussed above.

Other safety parameters

Integrated safety analyses

No evidence of increased risk of SVCD was observed in the Dengue Group compared to the Control Group during the 25-month observation period of active surveillance in each of the 3 efficacy studies overall or in the pooled analysis: 27 SVCD cases were reported in subjects 9 to 16 years who received at least 1 injection in the efficacy studies (4 in the Dengue Group and 23 in the Control Group) with a RR of 0.087 (95% CI: 0.02; 0.25) showing a statistically significant reduction of SVCD cases in the Dengue Group compared to the Control Group in this population during the Active Phase. Additionally, there was no evidence of increase in severity of SVCD cases based on the review of clinical outcomes and hospitalisation rates.

Long-term safety follow-up

Long-term follow-up 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.

At the time of submission, the following data from on-going long-term follow-up are available from the efficacy studies:

- Study CYD57: full data from the first 2 years of Hospital Phase (Hospital Phase Year 1 and Year 2, that is, 2 and 3 years after the end of the Active Phase in Study CYD23) and preliminary data from the third a and fourth years of Hospital Phase (cut-off date on 13 August 2015).
- Studies CYD14 and CYD15: full data from the first year of Hospital Phase (Hospital Phase Year 1, that is, 2 years after the end of the Active Phase in Studies CYD14 or

CYD15 and 3 years after the last injection) and preliminary data from the second and third years of follow-up (Hospital Phase Year 2 and Year 3) (cut-off date on 13 August 2015).

SAEs

No safety concerns were identified during long-term follow-up of all studies having a long-term follow-up (cut-off date on 1 September 2015), as no evidence of excess of any specific SAEs were reported. In particular, no related SAEs were reported in the Dengue Group.

Hospitalised VCD cases during completed years

The incidence of hospitalised VCD cases during the long term follow-up of the efficacy studies (Studies CYD14, CYD15 and CYD57) was assessed by age group in each individual study, that is, in subjects aged 2 to 5 years, 6 to 11 years and 12 to 14 years, as applicable.

The analyses from the Hospital Phase in Study CYD14 showed a higher incidence of hospitalised VCD cases in the Dengue Group compared to the Control Group in subjects aged 2 to 5 years at enrolment. The annual incidence rate of hospitalised VCD cases was 1.0% in the dengue group and 0.1% in the placebo group representing a relative risk (RR) of 7.454 (95% CI: 1.15; 313.80). In subjects aged 6 years and above at enrolment, the RR of hospitalised VCD cases was below 1 during the first year of the Hospital Phase, and this RR decreased with increasing age (RR < 1: 0.627 (95% CI: 0.22; 1.83) in subjects aged 6 to 11 years and RR < 1: 0.249 (95% CI: 0.02; 1.74) in subjects aged 12 to 14 years).

In the long term follow-up of the Phase IIb efficacy study (Study CYD57, with subjects aged 4 to 11 years at inclusion in Study CYD23); RR of hospitalised VCD cases varied each year and according to serotypes distribution and age groups. As for CYD14, the RR fluctuated over time in young age groups (4 to 5 year-old subjects), with RR of 2.443 and 0.814 the first and second years of hospital phase, respectively, while RR remained consistently below < 1 in older age groups (6 to 11 year-old subjects).

Conversely for Study CYD15, the analyses from the first year of Hospital Phase did not show any difference of incidence of hospitalised VCD cases between the CYD dengue and control groups. The annual incidence rate of hospitalised VCD cases in 9 to 11 year-old subjects was 0.2% in the dengue group and 0.3% in the placebo group representing a relative risk (RR) of 0.554 (95% CI: 0.20; 1.54). Similarly, the annual incidence rate of hospitalised VCD cases in 12 to 16 year-old subjects was < 0.1% in the dengue group and 0.2% in the placebo group representing a relative risk (RR) of 0.501 (95% CI: 0.13; 1.87).

To further inform the benefit/risk ratio of the CYD dengue vaccine, breakdown analyses were performed at different age cut-offs. The cut-off below and above 9 years of age was chosen defining different age groups: (i) subjects aged between 2 and 8 years and (ii) subjects aged between 9 and 14 years). The results in Studies CYD14 and CYD57 were compared to that observed in subjects from 9 years of age included in CYD15 where RR was consistently < 1 during the first year of Hospital Phase.

The incidence of hospitalised VCD cases during the first 2 years of the Hospital Phase in Study CYD57 and during the first year of the Hospital Phase in Studies CYD14 and CYD15 is presented with the cut-off at 9 years of age. In both CYD14 and CYD57, the analysis shows a lower RR in subjects aged 9 to 14 years compared to children aged 2 to 8 years. The RR in subjects aged 9 to 14 years in CYD14 (0.572) was similar to RR in subjects aged 9 to 16 years measured in CYD15 (0.533).

When comparing RR against hospitalised VCD cases in subjects aged 9 to 14 years during the Active Phase in CYD14 and CYD15 (0.185 and 0.197, respectively), to the RR during the first year of Hospital Phase (0.572 and 0.533, respectively), there appears to be a trend toward a higher risk of hospitalised VCD cases in this age group. However, when considering the cumulative data collected during the Active Phase and the first year of Hospital Phase in subjects aged 9 to 14 years included in Studies CYD14 and CYD15, the

RR during the entire study remained significantly < 1 with a value of 0.273 (95% CI: 0.14; 0.50) and 0.284 (95% CI: 0.18; 0.44), respectively. These results are in favour of a decreased risk of hospitalised VCD cases in the Dengue Group throughout the studies. The same trend was observed in CYD57, with RR in subjects aged 9 to 11 years significantly < 1 during the entire study (0.290 (95% CI: 0.13; 0.62)).

Clinical signs and symptoms of hospitalised VCD cases: Hospital versus Active Phase

Hospitalised VCD cases observed during the first year of Hospital Phase in the Dengue Group did not show a different clinical profile in terms of severity compared to the Control Group in the Hospital Phase and also compared to the Active Phase. The mean duration of fever, clinical symptoms, and hospitalisation was similar in the 2 treatment.

Viraemia levels in hospitalised VCD cases: Hospital versus Active Phase

Case viraemia levels were similar during the Active and the Hospital Phases. No increase of viraemia was observed in CYD dengue vaccine recipients compared to placebo recipients. *In vitro* and clinical data available so far indicates that CYD vaccination does not increase post-dengue disease viraemia and thus, dengue severity.

Hospitalised VCD cases during uncompleted years (preliminary data)

Study CYD57 Year 3 and Year 4 Hospital Phase, Study CYD14 and Study CYD15 Year 2 and Year 3 Hospital Phase are incomplete years (data collection is still ongoing or data are still unlocked). Therefore, data from these incomplete years are preliminary data obtained from non-validated databases and not analysed as per pre-planned interim analysis.

Preliminary data showing the number of hospitalised VCD cases collected up to the 13 August 2015 during the ongoing follow-up of Studies CYD14, CYD15 and CYD57 show to date:

- In Studies CYD14 and CYD15, there were less VCD cases reported in the Dengue Group than in the Control Group.
- In Study CYD57 during ongoing Year 4 Hospital Phase, there were 7 cases reported and all were in the Dengue group. In Studies CYD14 and CYD57, there were more VCD cases reported in the Dengue Group compared to the Control group in subjects < 9 years of age.
- Severe VCD Cases during Completed Years in Study CYD14, among the 40 hospitalised VCD cases reported during the first year of the Hospital Phase, 12 were assessed as clinically severe: 11 in the Dengue Group and 1 in the Control Group. As observed with hospitalised VCD cases, when considering the age group at enrolment, during the first year of the Hospital Phase, there was an unexplained difference of hospitalised SVCD cases between the Dengue Group and the Control Group in children aged 2 to 5 years (6 cases) and 6 to 11 years (5 cases). However RR was not calculated because there was no case in the Control Group. This difference between treatment groups was not seen in adolescents aged 12 to 14.
- In subjects aged 9 to 14 years, the RR against hospitalised SVCD cases during the first year of the Hospital Phase in Study CYD14 was 1.5 with a large CI which did not allow drawing definitive conclusions. However, during the entire study, the RR against hospitalised SVCD cases was < 1 and statistically significant (95% CI: 0.06; 0.71), indicating a decreased risk of SVCD cases in the Dengue Group compared to the Control Group. In Study CYD15, the RR against hospitalised SVCD cases during the first year of the Hospital Phase was 0.300 (95% CI: 0.05; 1.54). Similarly to CYD14, the RR against hospitalised SVCD cases during the entire study was < 1 and statistically significant (95% CI 0.02; 0.33), indicating a decreased risk of SVCD cases in the Dengue Group compared to the Control Group.

Post-marketing data

There is none available yet as the first regulatory authorities (Mexico, the Philippines and Brazil) only granted marketing authorisation to the CYD dengue vaccine in December 2015.[60]

Evaluator's conclusions on safety

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. Approximately 28,900 subjects aged 9 months through 60 years were randomised in 22 trials, to receive at least one injection of the tetravalent CYD dengue vaccine, regardless of the formulation and schedule. The database including the 22 studies in the pooled/integrated analysis should allow 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.

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 also found to be similar to that of comparator vaccines, i.e., different licensed vaccines mainly used as benefit vaccines or as part of the vaccination schedules of the age groups.

Solicited reactions were reported in a majority of subjects, regardless of age. Most of the solicited reactions within 7 days (injections site reactions) or 14 days (systemic reactions) were Grade 1 and resolved spontaneously within 1 to 3 days of onset. Immediate systemic AEs within 30 minutes after any injection as well as AEs leading to discontinuation occurred in 1.6% in adults or less (0.2% to 0.6%) in the younger subjects.

The incidence of unsolicited non-serious ARs was low in adolescents, children and toddlers (0.4% to 2.5%) but more frequently reported in adults (approximately 12%). No clusters of ARs were observed in any of the age groups. Grade 3 unsolicited non-serious ARs were reported by low proportions of subjects (1.3% or less) depending on the age group. Most unsolicited non-serious AEs and ARs were of Grade 1 and resolved spontaneously.

The incidence of solicited systemic reactions and unsolicited non-serious AEs and ARs tended to decrease with subsequent injections, while the incidence of solicited injection site reactions was similar after each injection. 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 Dengue Group, SAEs assessed as related to the study vaccine occurred in 7 subjects within 28 days of injections, 2 adults (headache and polymyalgia rheumatica), 1 adolescent (urticaria), and 4 children (ADEM, asthma, acute polyneuropathy, tension headache). None was reported in infants and toddlers. One SAE (blighted ovum) in an adult, assessed as related to the study vaccine, occurred between 28 days and 6 months after the injection. One SAE (convulsion) in a child was assessed as related to the study vaccine by the sponsor.

[60] After being granted market authorisation in The Philippines on 22 December 2015, the licence was subsequently suspended until 2 January 2019.

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. They were isolated events and for ADEM, acute polyneuropathy and convulsion, no vaccine viruses were isolated from the subjects. 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.

Less than 6% (38 subjects out of 683) subjects had vaccine viraemia after administration of the CYD dengue vaccine. In each case, vaccine viraemia recorded was low, i.e. very close to the LLOQ value, and no safety concerns were observed in these subjects.

The analysis of AESIs showed no particular concerns in terms of allergic reactions. In addition, very few potential allergic reactions were rated as Grade 3 or serious. No events of viscerotropic or neurotropic disease were observed after administration of the CYD dengue vaccine was observed in any studies. Serious dengue disease events did not raise any safety concern.

Three abnormal pregnancy outcomes were observed in 22 women who were exposed to the CYD dengue vaccine during their pregnancy (death in utero, stillbirth and blighted ovum). In all cases risk factors were identified. Furthermore, no difference in abnormal pregnancy outcomes was observed between the Dengue Group and the Placebo Group. No data on lactation were collected.

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 Latin America regions, and there was no excess of SVCD in the Dengue Group compared to the Control Group in the 2 endemic regions AP and Latin America. During the long-term follow-up (from Year 2 post last injection and beyond), no SVCD were reported in the non-efficacy Phase I/II Studies CYD05, CYD22 and CYD28. In the 3 efficacy studies (Studies CYD57, CYD14 and CYD15), based on validated data collected during the long-term safety follow-up (Year 1 and Year 2 Hospital Surveillance for CYD57 and Year 1 Hospital Phase for Studies CYD14 and CYD15), results per age group showed that, there was no evidence of excess of hospitalised VCD cases including SVCD cases in the Dengue Group compared to the Control Group in the subjects from 9 years of age, while there was a trend towards a higher incidence of hospitalised VCD and SVCD cases in subjects aged below 9 years.

There is no evidence, clinically, immunologically or virologically, that the disease in the Dengue Group is different to that observed with wild-type infection in Control Group. At the time of cut-off date for Hospital Surveillance/Phase data presentation (13 August 2015), preliminary data collected in Studies CYD57, CYD14 and CYD15 during uncompleted years of long term follow-up show the same trend, that is, a favourable benefit/risk ratio in subjects aged 9 years-old and above but an increased risk of hospitalised VCD including severe in subjects below 9 years of age.

First round benefit-risk assessment

First round assessment of benefits

Table 9: Assessment of benefits

Benefits	Risks and Uncertainties
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. Around 500,000 hospitalisations are reported each year, and around 20,000 cases would result in death. School age children and young adults represent a population at significant risk of dengue disease in endemic countries. This vaccine appears to have efficacy in decreasing the incidence of dengue infection, associated hospitalisations and deaths. Two pivotal Phase III efficacy studies were conducted during the clinical development of the CYD dengue vaccine: CYD14 and CYD15. 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	Considering the efficacy of the CYD dengue vaccine in populations from 9 years, VE was demonstrated in both 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 (FASE) during the first 25 months of the studies. 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 and high efficacy was observed for serotypes 3 and 4. In dengue non-immune subjects at baseline, VE was inconclusive due to the limited number of cases in individual studies. The meta-analysis pooling results from CYD14 and CYD15 showed an effect with a VE estimate at 52.5% (95% CI: 5.9; 76.1); indicating a benefit in this population. The data generated is based on a 3-dose schedule administered 6 months apart. It was not possible to explore the efficacy of only 1 or 2 injections over a long period of time although efficacy was observed between injections. Vaccine efficacy was influenced by several factors. At the study level, the distribution of serotypes in the region or country at the time of the clinical study influenced efficacy outcomes: when serotype 2 predominated, overall efficacy was lower. At an individual level, the subjects' age at vaccination, previous exposure to dengue (immune status to dengue at baseline), and level of the response to the vaccine all had an effect on efficacy outcomes. Age can be considered as a key factor in predicting VE, with VE increasing with age increase. The efficacy data described is based on 25-month follow-up of the Active Phase of the 2 Phase III efficacy studies (up to 13 months after completion of the 3-dose vaccine

Benefits	Risks and Uncertainties
	regimen). During this time period, efficacy persisted with no evidence of waning. Although efficacy has not been evaluated in adults, immunogenicity data from studies conducted in adults aged 18 to 45 years in AP (CYD22, CYD47) suggest that adults have a high level of seropositivity and respond well to the vaccine schedule used in the efficacy studies. Indeed, post- injection 3 Ab levels are generally higher than those seen in CYD14 and CYD15 where efficacy was demonstrated. This 'bridging' of immunogenicity data from the efficacy studies to immunogenicity data in adults aged 18 to 45 years showed that levels of titres should be predictive of protection in this population supporting the indication for prevention of dengue disease in an adult aged 18 to 45 years living in endemic area. Immunogenicity bridging data was not available for the 46 to 60 years old population in endemic regions, but the Applicant's conclude that 46 to 60 years adults in endemic regions would have a comparable safety and immunogenicity profile to the 18 to 45 years population in the same endemic regions similarly as what is observed in non-endemic regions. In addition the immune response in terms of GMTs is higher in endemic regions than in non-endemic regions. Therefore, 46 to 60 years adults in endemic regions are expected to have a similar VE than 18 to 45 years adults in the same regions, which is considered to be similar to VE demonstrated in adolescents. Although this data is not yet available and is basely solely on theoretical extrapolation. At the time of the current application, no data on the long-term efficacy of the vaccine is available in the target population for use. Long-term protection from dengue will be evaluated through Post Approval Effectiveness studies with a follow-up of 5 years as described in the RMP. Analysis from data collected in subjects from 9 years of age in CYD14 and CYD15 during the first year of Hospital Phase and from the first 2 years of CYD57 (long-term follow-up of CYD23) showed no trend to increased risk of hospitalised (severe and non-severe) VCD cases. No studies to evaluate the value of a booster dose have been conducted to date. However administration of a booster dose will be

Benefits	Risks and Uncertainties
	evaluated in follow-up studies from Phase II in endemic countries in Latin America and AP, in subjects aged 9 years and above at inclusion in the Phase II studies, who will be asked to participate to new studies to receive a booster injection around 5 years after the last injection (CYD63 and CYD64).

First round assessment of risks

Table 10: Assessment of risks

Risks	Strengths and Uncertainties
Risk of AEs, SAES, ADRs related to vaccine. Risk of unacceptable reactogenicity profile. Possible increase in VCD cases requiring hospitalisation (severe and non-severe) in subjects aged 2 to 8 years (as seen in CYD14 and CYD23) Hospital Phase, that is, potential risk of increase in severity of dengue disease in this age group.	The safety data is available for approximately 28,600 subjects aged 9 months to 60 years who 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 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 hospitalised VCD for 5 years post-injection 3 with the provision of regular safety reports in an ongoing basis. No safety concerns were identified from the review of SAEs from the long-term follow-up of the CYD dengue vaccine clinical studies. In particular no related SAEs have been reported during the long-term follow-up, although it is still continuing. The methodology and outcome measures for the safety database were age 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

Risks	Strengths and Uncertainties
	that have been studied and also similar when compared to placebo. During the Active Phase observation period of the Phase III efficacy studies, rates of severe VCD cases and hospitalised VCD cases in subjects from 9 years were significantly lower in the vaccinated treatment groups. From the analysis of the first year of Hospital Phase of CYD14 and CYD15, there is no increased risk of hospitalised VCD cases (including severe) in the 9 to 16 year-old population, nor in the first 2 years of Hospital Phase of CYD57 in subjects aged 9 to 11 years. This continues to be closely monitored in the long-term follow- up of CYD14, CYD15 and CYD57 (Hospital Phase) which will continue for 5 years. Post-approval effectiveness studies will also address the potential risk of increased severity of disease for the CYD dengue vaccinees in the target populations for licensure with studies designed with 5 years duration of follow-up.

First round assessment of benefit-risk balance

Data from the clinical development of the CYD dengue vaccine has shown that with a 3 dose regimen administered 6 months apart the vaccine is efficacious at the prevention of dengue disease in the subjects aged 9 to 16 years. Efficacy was observed against each of the 4 serotypes with high efficacy seen against severe VCD cases and hospitalised VCD cases over a 25 month observation period. The high post-injection titres seen in adults living in endemic areas in Asia allow us to theoretically bridge immunologically to an adult population.

There are no safety concerns surrounding the reactogenicity and AE profile of the candidate vaccine in the cumulative data provided. Overall, in the long-term follow-up data during the Hospital Phase across the 3 studies, no evidence of increased severity of dengue disease or increase in frequency of hospitalised Dengue cases has been observed in the 9 to 16 year olds. From this, the benefit/risk balance of the CYD dengue vaccine is positive.

First round recommendation regarding authorisation

The evaluator would recommend registration for an indication 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.

Second round evaluation

For details of the second round evaluation including the issues raised by the evaluator (Clinical questions), the sponsor's responses and the evaluation of these responses please see Attachment 2.

Second round benefit-risk assessment

There was no second round evaluation as no new clinical data was the submitted by the sponsor in response to questions raised.

VI. Pharmacovigilance findings

Risk management plan

Summary of RMP evaluation[61]

- The sponsor has submitted EU-RMP version 1.0 (29 January 2016; DLP 13 August 2015) and ASA version 1.0 (24 May 2016) in support of this application. In its Section 31 response, the sponsor has submitted EU-RMP version 2.0 (dated 10 January 2017, DLP 18 September 2016) and ASA version 1.1 (dated 31 January 2017).
- The proposed Summary of Safety Concerns and their associated risk monitoring and mitigation strategies as described in EU-RMP version 2 are summarised below.

[61] *Routine risk minimisation* activities may be limited to ensuring that suitable warnings are included in the product information or by careful use of labelling and packaging. *Routine pharmacovigilance* practices involve the following activities:

- All suspected adverse reactions that are reported to the personnel of the company are collected and collated in an accessible manner;
- Reporting to regulatory authorities;
- Continuous monitoring of the safety profiles of approved products including signal detection and updating of labelling;
- Submission of PSURs;
- Meeting other local regulatory agency requirements.

Table 11: Summary of safety concerns

Summary of safety concerns		Pharmacovigilance		Risk Minimisation	
		Routine	Additional	Routine	Additional
Important identified risks	Allergic (including Anaphylactic) Reactions	ü	ü**	ü	ü^
Important potential risks	YF vaccine-associated viscerotropic disease (YEL-AVD)	ü	ü**	–	–
	YF vaccine-associated neurotropic disease (YEL-AND)	ü	ü**	–	–
	Increase in the severity of dengue disease from the start of vaccination	ü	ü**	ü	ü^
	Waning protection against dengue disease over time	ü	ü**	ü	ü^
Missing information	Safety in immunocompromised subjects (including subjects with congenital or acquired immune deficiency, or with HIV infection with impaired immune function).	ü	ü**	ü	ü^
	Safety profile of inadvertent use in pregnant or lactating women.	ü	ü**	ü	ü^
	Co-administration of CYD dengue vaccine with HPV vaccine or booster dose of Tap vaccine	ü	ü**	ü	–

^Risk minimisation activities only for Australia. ** A number of activities and studies are planned in highly-endemic areas; therefore no Australian patients are/will be included in these studies.

- Additional pharmacovigilance activities include five ongoing and three planned studies mainly in highly endemic areas addressing the Safety Concerns outlined in the table above. Enhanced pharmacovigilance activities are also planned mainly for some countries in South East Asia and Latin America where PV practices in collecting adverse event data and sharing with the Health Authorities are still under development.
- Enhanced pharmacovigilance activities are also proposed in the EU RMP but not the ASA as these enhanced activities are considered routine in Australia.
- An additional risk minimisation activity (Health Care Professional Checklist) is proposed for Australia only for the Safety Concerns indicated in table above.

New and outstanding recommendations from second round evaluation

There is one outstanding recommendation:

- Renal and hepatic metabolism should be included under Missing Information in the Summary of Safety Concerns in the ASA.

It is noted that should the sponsor seek to extend the indications to allow marketing of Dengvaxia in Australia, the RMP would need to be revised to address the issues raised in this report.

Advice to the delegate

The proposed PI has a paragraph regarding the excipients used in the vaccine. In the SmPC excipients are listed under a heading 'List of excipients: Powder and Solvent for reconstitution'. This makes it easier to see what the excipients are, and highlights that the vaccine contains 'Essential amino acids including L-Phenylalanine' and 'D-Sorbitol (E420)' which is important in those with phenylketonuria or fructose intolerance. The Delegate may wish to consider if this formatting is appropriate for the Australian PI.

In the first round RMP evaluation it was recommended that an explanation of 'endemic' for example, regularly found among particular people or in a certain area) would be helpful in the CMI. The Sponsor has indicated that they would consider revisions to the CMI based on the Delegate's advice on the indication.

Proposed wording for conditions of registration

Any changes to which the sponsor has agreed should be included in a revised RMP and ASA. However, irrespective of whether or not they are included in the currently available version of the RMP document, the agreed changes become part of the risk management system.

The suggested wording is:

- EU-RMP (version 2.0, dated 10 January 2017, data lock point 18 September 2016) with ASA (version 1.1, dated 31 January 2017) to be revised to the satisfaction of the TGA, must be implemented as a condition of registration.

VII. Overall conclusion and risk/benefit assessment

The submission was summarised in the following Delegate's overview and recommendations:

Quality

CYD dengue vaccine is a tetravalent, live attenuated viral vaccine. Each CYD dengue virus serotype was obtained separately from parental yellow fever 17D virus (YF-17D) and wt dengue viruses 1 to 4 via recombinant DNA technology.

CYD dengue viruses were constructed by replacing the sequence encoding the prM and E structural ('coat') proteins in YF-17D virus genome by those encoding for the homologous sequences of the four wt dengue serotypes 1 (PUO-359/TVP-1140), 2 (PUO-218), 3 (PaH881/88), and 4 (1228/TVP-980). No additional sequences were added. The immunising antigens are PrM and E proteins from dengue virus serotypes 1 to 4.

The manufacturing process involves dengue virus seed lots, Vero cell banking system and control of drug substance and drug product. Overall, sufficient evidence has been provided to demonstrate that the risks related to the manufacturing quality of Dengvaxia Dengue tetravalent vaccine (live, attenuated) have been controlled to an acceptable level. There are no objections to the registration of this product from sterility; endotoxin, container safety and viral safety related aspects. The quality summary comments that 'It is to be noted that 'Particles and filaments' at both DS and DP stages on CYD dengue samples were observed and were considered the result from the aggregation of endogenous proteins of intrinsic characteristics (that is, proteins of Vero cell origin). Presence of 1 particle/dose in Dengvaxia vaccine is considered as an intrinsic characteristic of CYD dengue vaccine'.

The evaluator recommends that there are no further objections to the registration of Dengvaxia Dengue tetravalent vaccine (live, attenuated). However, it should be noted that the Drug Product contains endogenous particles/filaments. There are no further concerns related to quality of these particles/filaments but safety signals of these particles/filaments are beyond this quality evaluation report.

Nonclinical

CYD vaccine induced neutralising antibodies against all 4 serotypes in non-human primates (NHPs), the antibody responses appeared durable for up to a year, the maximum period studied. Comparison of monovalent and tetravalent vaccine indicated interference between serotypes. A 3-dose regimen was the most successful in terms of balancing neutralising antibody responses to all 4 serotypes.

Dengue protection studies were conducted in NHPs. A major limitation of the studies was the failure of NHPs to develop dengue disease, hence protection was assessed in terms of reduction or absence of viraemia. Most (22/24) vaccinated NHPs were fully protected (no viraemia) against a moderate SC challenge, and partially protected against a high-dose IV challenge. Several monkeys were protected despite low neutralising antibody titres, possibly due to cell-mediated immunity.

Cross protection in vitro was shown with monkey and human sera against representative panels of wild type DENV.

There is a theoretical risk of sensitisation due to CYD vaccination which was investigated in terms of enhancement of viraemia. NHPs do not develop dengue disease after SC inoculation No enhanced viraemia was observed in any of the NHP studies. However, the validity of the model is uncertain.

No nonclinical data were submitted on cross-reactive antibodies to other flaviviruses.

Nonclinical biodistribution, toxicity and neurovirulence studies in NHPs showed that the vaccine viruses are highly attenuated, with a transient minimal to slight inflammatory reaction at the injection site being the only finding. Viraemia was low and transient, no virus shedding was detected. Genetic stability studies indicated that reversion to virulence by back mutation or recombination is very unlikely.

Reproductive toxicity studies in mice and rabbits did not show any direct adverse effects on the fetus, however due to limitations of the models a pregnancy category of B2 is recommended.

There are no nonclinical objections to registration, provided that efficacy and the potential for sensitisation to flavivirus disease have been satisfactorily addressed in clinical studies.

Clinical

As of December 2015, the CDP includes 25 clinical studies, completed (21) or on-going (4): 5 Phase I, 14 Phase II and 6 Phase III studies.

A total of more than 41,000 subjects have been enrolled in clinical studies including more than 28,500 subjects from 9 months through 60 years of age exposed to at least one injection of the final tetravalent CYD dengue vaccine formulation, regardless of the administration schedule. Among these subjects, 21,215 subjects were aged 9 through 60 years and received at least one injection of the final formulation of the CYD dengue vaccine, regardless of the schedule.

Pharmacology

The pharmacodynamic profile for the CYD dengue vaccine was defined by its immunogenicity profile.

Immunological assay methods

The PRNT assay was used to assess the humoral immune response of the CYD dengue vaccine through the measurement of neutralising Ab at varying time points. The PRNT was performed by the applicant's laboratory, for all studies that assessed the final formulation

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

- GMTs expressed in reciprocal of dilution (1/dil) for each serotype.
- GMTRs from baseline to post-vaccination for each serotype.
- Seropositivity rate, defined as the proportion of subjects with a neutralising Ab titre ≥ 10 (1/dil). This level also corresponds to the lower limit of quantification (LLOQ) of the PRNT assay.

To further characterise the immune response induced by the CYD dengue vaccine and as recommended in the WHO and EMA guidelines, cell-mediated immunity was also assessed in some studies in adolescents and adults in endemic and non-endemic regions (Studies CYD04, CYD10, CYD11 and CYD28).

Dose finding studies

There were a number of Phase I and II studies performed to examine the dose and scheduling of the CYD vaccine. Eight clinical studies (5 Phase I studies and 3 Phase II studies) were conducted for that purpose.

Study CYD01 assessed safety and immunogenicity of a single dose of monovalent chimeric dengue 2 vaccine (ChimerivaxTM-DEN2) containing 5 or 3 log10 plaque forming units (PFU)) and showed that one dose of monovalent chimeric dengue 2 vaccine induced a satisfactory immune response against serotype 2 and low seropositivity rates to the other 3 serotypes (in YF non-immune subjects), confirming the need for a tetravalent vaccine.

A tetravalent vaccine against the 4 serotypes was tested in Study CYD04 and showed satisfactory safety and immunogenicity profiles in flavivirus (FV) non-immune adults, and, in Studies CYD05 and CYD06, in different age groups (2 to 45 years) and FV backgrounds. The immunogenicity response varied across populations due to co-factors (that is, age, baseline status, region). Seropositivity rates against all 4 serotypes after 3 injections of a tetravalent formulation ranged from 39.1% (Study CYD04, FV non-immune adults) to 85.0% (Study CYD05, FV immune adults, adolescents, and children).

These Phase I studies together with 3 Phase II Studies CYD10, CYD11 and CYD12, provided data on safety and immune response induced by several formulations of the vaccine and different schedules of vaccination.

The choice of a tetravalent formulation was confirmed by the use of sequential or simultaneous bivalent formulations in CYD11, which did not improve the immune response compared to the tetravalent formulation.

The schedule with 3 injections at 6 month intervals was further confirmed with subsequent studies (Studies CYD13, CYD22, CYD 24, CYD28, CYD30 and CYD47) conducted in different settings (age, baseline FV status) using the same Phase II lots of the final formulation with the chosen schedule.

A co-administration Phase II study (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.

Efficacy

The evaluation of efficacy of the CYD dengue vaccine is based on the vaccine efficacy (VE) observed in the 2 pivotal Phase III efficacy Studies CYD14 and CYD15. Supportive clinical data were obtained from Study CYD23, a Phase IIb proof of concept efficacy study. The Phase III efficacy studies were randomised, placebo-controlled, observer- blinded and stratified by age. Study CYD23 was randomised, observer blinded, and involved rabies vaccine/placebo comparators.

Studies CYD14 and CYD15 are described together.

Two Phase III efficacy studies, each statistically powered to independently demonstrate efficacy, were designed and prepared to be carried out in parallel in 10 endemic countries: Study CYD14 (5 countries in Asia Pacific, 2 to 14 year old children) and Study CYD15 (5 countries in Latin America, 9 to 16 year old children and adolescents).

The main objective of the Phase III efficacy studies was to demonstrate VE of the CYD dengue vaccine in preventing virologically confirmed dengue (VCD) cases, in accordance with WHO guidelines on dengue vaccine evaluation. The primary endpoint in efficacy studies was defined as: 'Symptomatic VCD cases' occurring more than 28 days after the third injection up to the end of the Active Phase.

The studies were divided into Active and Hospital phases. During the Active Phase, surveillance was designed to maximise the detection of symptomatic confirmed dengue. For each subject, the Active Phase of dengue case detection began after the first injection (Dose 1) and was expected to continue until 13 months after the third injection (Dose 3). The Hospital Phase began after the Active Phase. Subjects with a febrile illness and requiring hospitalisation were screened for dengue. This phase is currently ongoing and will continue until trial completion. In this phase, there is a minimum frequency of one contact every 3 months and surveillance of identified non-study healthcare sites is being performed.

Study CYD14 was conducted at 11 sites across Indonesia, Malaysia, Thailand, the Philippines, and Vietnam (2 to 3 sites in each country). Study CYD14 was conducted June 2011 to December 2014. Subjects were randomised in a 2 to 1 ratio to 2 groups:

- CYD Dengue Vaccine Group (N = 6851): CYD dengue vaccine at 0, 6 and12 months
- Control Group (N = 3424): Placebo at 0, 6 and12 months.

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.

Study CYD15 was conducted at 22 sites across Brazil, Colombia, Honduras, Mexico, and Puerto Rico. Study CYD15 was conducted between June 2011 to December 2014.

Subjects were randomised in a 2 to 1 ratio to 2 groups:

- CYD Dengue Vaccine group (N = 13,917) CYD dengue vaccine at 0, 6 and 12 months
- Control Group (N = 69,586): placebo at 0, 6 and12 months.

Immunogenicity and reactogenicity were assessed in a subset of subjects to enable the generation of country-specific data on reactogenicity, immunogenicity, and baseline dengue and yellow fever (YF) antibody (Ab) levels.

Study methods are further described.

In Study CYD14, a total of 10,275 subjects were randomised: 6851 in the CYD Dengue Vaccine Group and 3424 in the Control Group. A total of 2000 subjects, 1336 in the CYD Dengue Vaccine Group and 664 in the Control Group, were included in the immunogenicity and reactogenicity subset.

Baseline data

Overall, 10,194 subjects (99.2%) completed the Active Phase of the study and 10,143 (98.7%) completed the first year of the Hospital Phase. The same percentage was observed in the subset for the Active Phase and for the Hospital Phase. A total of 10,272 subjects (3 were not vaccinated) were included in the full analysis set for efficacy (FASE) and 10,060 subjects were included in the per-protocol analysis set for efficacy (PPSE).

In the immunogenicity and reactogenicity subset, the proportion of dengue immune subjects at baseline (neutralising Ab response ≥ 10 (1/dil) using Dengue PRNT50) 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. Differences in terms of dengue status at baseline were observed across countries: from 47.8% of dengue immune subjects at baseline in Malaysia to 80.8% in Indonesia.

In the PPSE, there were similar percentages of female (51.5%) and male subjects (48.5%). Overall, 24.0% of the subjects were in the age group 2 to 5 years old, 53.3% in the age group 6 to 11 years old, and 22.8% in the age group 12 to 14 years old. The mean age at enrolment was 8.8 years. The demographic characteristics were well-balanced between the treatment groups.

In Study CYD15, a total of 20,869 subjects were randomised: 13,920 in the CYD Dengue Vaccine Group and 6949 in the Control Group. Overall, 19,921 (95.4%) completed the Active Phase of the study and 19,921(95.4%) completed the first year of the Hospital Phase.

A total of 18,834 subjects were included in the PPSE.

In the PPSE, there were similar percentages of female (50.3%) and male subjects (49.7%). 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. Overall, 79.4% of the subjects were dengue-seropositive at baseline. Percentages were similar in both treatment groups. 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%). Dengue and YF seropositivity rates were similar in both age groups, although slightly higher in the older age group (74.9% and 77.1% for dengue and YF respectively in the 9 to 11 years age group versus 83.8% and 82.3% for dengue and YF, respectively, in the 12 to 16 years age group).

Results for the primary efficacy outcome for both studies

During the Active Phase of Study CYD14, all 4 serotypes were circulating in the 5 countries and within each individual country with a different serotypes distribution. The density incidence of virologically-confirmed dengue (VCD) in children in the Control Group was 4.7% during the 25-month active surveillance period and 4.1 % during the post-dose 3 period (that is, from 28 days post-dose 3 until the end of 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.

In Study CYD14, in the PPSE, a total of 250 subjects reported at least 1 VCD case from 28 days post-injection 3 to the end of the Active Phase. The overall primary estimate of VE against VCD post- injection 3 due to any serotype was 56.5%. The primary objective was met with a lower bound of 95% CI above 25%. This result was confirmed in the FASE population, including all VCD cases that occurred during the first 25 months of the study in all subjects having received at least one injection, with a VE of 54.8%. In subjects aged 9 to 14 years, VE was confirmed and was higher (67.8% after at least one injection) than in the overall population.

In Study CYD15 the incidence of dengue in the Control Group was 2.9% during the Active Phase. All 4 serotypes were detected during the Active Phase of the study. In Study CYD15, in the PPSE, a total of 397 subjects reported at least 1 VCD case from 28 days post-injection 3 to the end of the Active Phase. The overall primary estimate of VE against VCD from 28 days post-injection 3 to the end of the Active Phase due to any serotype was 60.8%. The primary objective was met with a lower bound of 95% CI above 25%. This result was confirmed in the FASE population, which included all VCD cases that occurred

during the first 25 months of the study in all subjects having received at least one injection with a VE of 64.7%.

For Studies CYP14 and CYD15 combined, overall, 55% to 65% VE was observed in preventing occurrence of VCD cases due to any serotype after at least one injection of the CYD dengue vaccine. Significant VE was also observed in preventing the occurrence VCD cases due to each serotype after at least one injection of the CYD dengue vaccine. This varied according to the serotype: moderate efficacy was observed for serotypes 1 and 2 and high efficacy was observed for serotypes 3 and 4. This finding was consistent across the endemic regions evaluated. VE estimates in subjects aged 9 to 16 years are summarised.

Results for other efficacy outcomes in Studies CYD14 and CYD15

VE by serotype

VE was analysed by serotype from 28 days post-injection 3 to the end of the Active Phase. VE is demonstrated for all 4 serotypes in Studies CYD14 and CYD15 after at least one injection. In the meta-analysis pooling results from Studies CYD14 and CYD15, VE estimate against each serotype was 54.7% for serotype 1; 43.0% for serotype 2; 71.6% for serotype 3 and 76.9% for serotype 4.

Severe VCD

In Study CYD14, IDMC assessment of severe dengue determined that the vaccine reduced the occurrence of severe VCD by 70% (based on 12 clinically SVCD cases in the Dengue Group and 20 clinically SVCD cases in the Control Group). The vaccine reduced the occurrence of DHF of any grade according to the WHO criteria by 80.0% (based on 8 DHF of any grade in the Dengue Group and 20 in the Control Group). In subjects aged 9 years and over, higher VE estimates were observed (90.9% for both IDMC and DHF VCD cases).

In Study CYD15, the occurrences of clinically SVCD cases as assessed by IDMC and of DHF of any grade according to the WHO classification were reduced by at least 95% (based on 1 clinically SVCD cases in the Dengue Group and 11 in the Control Group, and based on 1 DHF of any grade in the Dengue Group and 10 in the Control Group). VE against clinically SVCD cases (as per IDMC definition) was also assessed by serotype. Few cases were reported but all four serotypes contribute to VE against SVCD, with no serotype predominant.

Hospitalised dengue cases

In Study CYD14, during the active phase, a total of 101 subjects, 40 in the CYD Dengue Vaccine Group and 61 in the Control Group, were hospitalised with VCD. Overall, a reduction of more than 67% of the incidence of hospitalised dengue cases due to any serotype in subjects receiving at least 1 dose was observed in CYD Dengue Vaccine Group as compared to Control Group (relative risk (RR): 0.328 (95% CI: 0.21; 0.50)).

Post-dose 3, 55 subjects (20 in the CYD Dengue Vaccine Group and 35 in the Control Group) were hospitalised with VCD. Overall, a reduction of more than 71% of the incidence of hospitalised dengue cases due to any serotype was observed in the CYD Dengue Vaccine Group as compared to the Control Group (RR: 0. 286 (95% CI: 0.16; 0.51)).

During the first year of the Hospital Phase, a total of 40 hospitalised VCD episodes due to any serotype were observed in 40 subjects: 27 in the CYD Dengue Vaccine Group out of 6778 subjects and 13 in the Control Group out of 3387 subjects, representing a RR against hospitalised VCD cases of the vaccinees compared to the Control Group of 1.038 (95% CI: 0.52; 2.19). The RR against hospitalised VCD cases during the entire study was 0.459 (95% CI: 0.32; 0.65) in favour of a decreased risk of hospitalised VCD cases in the CYD Dengue Vaccine Group.

In Study CYD15, during the active phase, a total of 60 subjects were hospitalised for a VCD case, that is, 17 in the CYD Dengue Vaccine Group and 43 in the Control Group. All serotypes. Overall, an 80.3% reduction of the incidence of hospitalised dengue cases due to any serotype in subjects receiving at least 1 dose was observed in CYD Vaccine Group, compared to the Control Group, as indicated by the relative risk (RR): 0.197 (95% CI: 0.11; 0.35). From 28 days post-Injection 3 to the end of the Active Phase, a total of 40 subjects were hospitalised for a VCD case. Overall, a reduction of the incidence of hospitalised VCD cases due to any serotype was observed in CYD Vaccine Group compared to the Control Group, as indicated by a RR of 0. 214 (95% CI: 0.10; 0.43).

During the first year of the Hospital Phase, 31 hospitalised VCD episodes due to any serotype were observed in 31 subjects: 16 in the CYD Dengue Vaccine Group out of 13,268 subjects and 15 in the Control Group out of 6630 subjects. The corresponding annual incidence rate of hospitalised VCD cases was 0.1% in the CYD Dengue Vaccine Group and 0.2% in the Control Group, representing a RR of the vaccinees compared to the Control Group of 0.533 (95% CI: 0.25; 1.16). A slight increase of the RR against hospitalised VCD cases during the first year of the Hospital Phase was observed when compared to the Active Phase. The RR against hospitalised VCD cases during the entire study (from D0 to the end of the first year of the Hospital Phase) was < 1: RR of 0.284 (95% CI: 0.18; 0.44), in favour of a decreased risk of hospitalised VCD cases in the CYD Dengue Vaccine Group compared to the Control Group.

Immunogenicity data

In Study CYD14, baseline seropositivity rates (percentages of subjects with neutralising Ab titres ≥ 10 (1/dil)) against at least 1 serotype were similar in both treatment groups.

At baseline, in the FASI, the percentage of subjects seropositive against at least 1, 2, 3 or 4 serotypes, were similar across the 2 treatment groups. Seropositivity at baseline against all 4 serotypes was 42.0% in the CYD Dengue Vaccine Group and 40.8% in the Control Group. In the CYD Dengue Vaccine Group, the percentages of subjects seropositive increased post-injection 2 and post-injection 3 (84.9% and 91.0%, respectively). One year post-injection 3, the percentage of subjects seropositive remained high as compared to post-injection 3 (72.0%). Two years post-injection 3, the percentage of subjects seropositive remained high (65.4%).

Overall, geometric means of titres (GMTs) at baseline were comparable across serotypes with a trend to higher GMTs for serotype 2. GMTs ranged from 25.3 (1/dil) for serotype 4 to 55.3 (1/dil) for serotype 2 in the CYD Dengue Vaccine Group and from 26.2 (1/dil) for serotype 4 to 62.1 (1/dil) for serotype 2 in the Control Group.

In the CYD Dengue Vaccine Group, GMTs at baseline, after the second and the third injection tended to be higher with age. Two years post-injection 3, GMTs were still higher as compared to baseline for all serotypes and for all age groups.

In the CYD Dengue Vaccine Group, GMTs per serotype post-injection 2 and post-injection 3 were higher in dengue immune subjects at baseline as compared to dengue non-immune subjects at baseline.

Study CYD23 is a Phase IIb PoC Efficacy study that provides supportive efficacy data. Study CYD23 was a randomised, observer blind, controlled, monocentre, Phase IIb trial which included 4002 subjects aged 4 to 11 years in Thailand. This study was conducted between March 2012 and September 2013. Long term follow-up is reported as Study CYD57.

Subjects were randomised in 2 cohorts to a CYD dengue vaccine group or to a control group. In cohort 1: 100 subjects were randomised to receive 3 injections (at 0, 6 and 12 months) of CYD dengue vaccine and 50 subjects were randomised to receive1 injection of rabies vaccine and two injections of placebo. After review of Day 14 safety data, the IDMC and sponsor recommended Cohort 2 to proceed. In cohort 2: 2569 subjects were

randomised to receive 3 injections of CYD dengue vaccine and 1283 subjects were randomised to receive 3 injections of placebo.

The active detection of dengue cases (that is, the Active Phase) started from the first injection until all subjects had been followed for at least 13 months after the third injection, on the condition that at least 27 cases of VC dengue had been detected and included in the per-protocol analysis set for efficacy (PPSE). Beyond this time point, the detection of hospitalised dengue cases up to 5 years after the last injection in addition to fatal and related SAEs is done through CYD57.

The primary objective was to assess the efficacy of dengue vaccine after three injections in preventing symptomatic VC dengue cases, regardless of the severity, due to any of the four serotypes in children aged 4 to 11 years at the time of inclusion on the condition that at least 27 cases of VC dengue had been detected and included in the PPSE.

Baseline results

A total of 4014 subjects were screened from which 4002 were enrolled and randomised (2669 subjects in the dengue group and 1333 subjects in the control group); 150 subjects were enrolled and randomised in Cohort 1 (100 subjects in the dengue group and 50 subjects in the control group) and 3852 subjects in Cohort 2 (2569 subjects in the dengue group and 1283 subjects in the control group).

The vast majority of subjects (95.7% of all subjects) present at V01 completed the Active Phase of the study. Approximately 96% subjects were included in the FASE and approximately 92% were included in the PPSE.

Overall, there were slightly more female (51.8%) than male subjects (48.2%) and the mean age was 8.17 years. All demographic characteristics were similar in both treatment groups in the biological, immunological and reactogenicity subsets.

Primary objective

After 3 injections of CYD dengue vaccine, the overall VE estimate was 30.2% (95% CI: -13.4, 56.6); the level of significance was not reached. A total of 78 VC dengue episodes were observed in 77 subjects after the completion of the 3 injections. Although this was more than the original estimate of 27 VC dengue cases, both the higher than estimated attack rate of dengue and a VE estimate that was lower than the 70% assumption resulted in the observed vaccine efficacy that did not reach statistical significance.

In this study, the primary estimate of VE was lower than anticipated and was not significant. 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) for which the vaccine is least effective.

Results for secondary objectives

Considering both WHO 1999 and IDMC severity assessments, a total of 5 severe VC dengue cases were identified during the Active Phase. Three were severe according to both WHO and IDMC severity assessments (1 in the dengue group and 2 in the control group. Moreover, there were no increases in the classic clinical signs of dengue such as bleeding, plasma leakage, or thrombocytopenia in vaccinees compared to controls. In conclusion, these results showed no increase of severe VC dengue in vaccinees as compared to controls.

Duration of clinical syndrome and hospitalisation

Independently from the number of cases observed in each group, there were no differences between vaccinees and controls with regard to the rate of hospitalisation or the duration of fever, clinical syndrome or hospitalisation. This demonstrates that breakthrough dengue infection in vaccinees was not clinically more severe than that

observed in control subjects. There is no evidence of any enhanced disease in vaccinees infected with dengue.

Immunogenicity results

Baseline seropositivity rates against each serotype were similar across serotypes for both treatment groups (ranged from 54.8% to 60.4% in the dengue group and from 45.5% to 58.2% in the control group). Seropositivity rates against each serotype increased after the first and second injections of CYD dengue vaccine, and were > 90% 28 days after the second injection. One year after the third injection of CYD dengue vaccine, the seropositivity rates remained > 80% against all 4 serotypes.

Four other Phase III clinical studies (CYD17, CYD29, CYD32 and CYD33) were also conducted:

- Study CYD17: Phase III Lot-to-Lot Consistency Study in Adults in Australia, Lot-to-Lot Consistency and Bridging Study of a Tetravalent Dengue Vaccine in Healthy Adults in Australia.
- Study CYD32: Phase III Study in Children in Malaysia, Safety and Immunogenicity of a Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 11 Years in Malaysia.
- The 2 other Phase III studies investigated concomitant administration with vaccines given to infants and toddlers below 2 years of age: YF co-administration with the first injection of CYD dengue vaccine from 12 months of age in Peru and Colombia (Study CYD29) and co- administration of DTacP-IPV booster with the second injection of the CYD dengue vaccine from 15 months of age in Mexico (Study CYD33).

Immunogenicity data

Immunogenicity results for clinical studies in endemic populations by age group are summarised and an integrated immunogenicity analysis is discussed.

Results show that Ab responses are higher in adults from endemic areas (Vietnam and India) than for areas of low endemicity (Singapore). Results in adults are also higher than in children and adolescents from the same endemic regions. In addition, GMTs in 9 to 17 year olds are similar or marginally higher in subjects from Latin America compared to AP regions depending on the specific serotype.

Dengue immune subjects at baseline had higher post-injection Ab responses than age matched dengue non-immune subjects. Since subjects aged 2 to 5 years show a much higher proportion of dengue non-immune subjects at baseline, as expected, they had lower Ab responses and lower efficacy. Subjects from endemic areas in the claimed population had the highest GMT while subjects in the 9 to 15 months old group had the lowest.

Immunogenicity data from studies conducted in adults aged 18 to 45 years in endemic areas (Studies CYD22 and CYD47) suggest that adults have a high level of GMTs and respond well to the vaccine schedule used in the efficacy studies.

Immunogenicity was assessed in 715 randomised adults aged 18 to 60 years in Australia in Study CYD17. The study had a primary lot-to-lot consistency objective and lot consistency was demonstrated for 3 Phase III lots and a Phase II lot. At baseline, 2.4% and 6.6% of subjects in pooled Dengue Group were FV and dengue immune, respectively; 5.3% and 8.8% of subjects in the Control Group, were FV and dengue immune, respectively. In the Control Group, dengue GMTs and seropositivity rates against each serotype did not appreciably change after the third injection. In the Dengue Group, PD3 GMTs were higher against serotype 3, 4 and 2 compared to serotype 1, ranging from 45.3 (1/dil) for serotype 2 to 111 (1/dil) for serotype 4, compared to 18 (1/dil) for serotype 1. After the third injection of the CYD dengue vaccine, more than 62% of subjects were seropositive against each serotype considered separately and 54.1% of subjects were seropositive against all 4 serotypes.

Ab persistence data are available post-injection 3 up to 5 years in CYD05, up to 4 years in Studies CYD22 and CYD28, up to 1 year in Study CYD23, and up to 2 years in Studies CYD14 and CYD15. Based on the data currently available on long-term follow up, a predictable decrease in the level of circulating antibodies (GMTs) against all four serotypes is observed 2 years after the third injection in all studies, regardless of the age group (the same trend was observed in adults, adolescents and children). However, overall these GMTs remain several folds higher than the baseline values. From 2 to 4 years after the third injection, the data available show a trend to a stabilisation of the GMTs, which still remain overall above baseline against all 4 serotypes.

Clinical safety

Methods for analysis of safety in clinical studies are described.

The safety database for the CYD dengue vaccine consists of all subjects who received at least one injection of the tetravalent CYD dengue vaccine containing approximately 5 $\log_{10}$ $CCID_{50}$ of each serotype administered with the final vaccination schedule, that is, 3 injections 6 months apart. Data were pooled and presented by age of study subjects: including adults (18 to 60 years), adolescents (12 to 17 years), children (2 to 11 years, further divided in 2 to 5 and 6 to 11 years), and infants and toddlers (below 2 years of age). To date, a total of approximately 28,600 subjects aged 9 months to 60 years have received at least one injection of the final formulation tetravalent CYD dengue vaccine. The database includes 22 studies in the pooled/integrated analysis.

A total of 4615 subjects aged 9 to 60 years (3068 were 9 to 17 years, and 1547 were adults aged 18 to 60 years) were included in the reactogenicity subset, in which solicited injection site and systemic reactions and unsolicited AEs were assessed.

Slightly less than one half of subjects 9 to 60 years 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 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 study Investigators, whereas in subjects aged 9 to 17 years, 2.2% of subjects had at least one unsolicited AE assessed as related to injection. Non-serious unsolicited 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. It was concluded there were no safety concerns related to the nature and frequency of unsolicited AEs. Similar trends were in adolescents (12-17 years), children (2 to 5 years and 6 to 11 years) and infants and toddlers.

Grade 3 solicited injection site reactions were reported in 0.7% of adults (18 to 60 years) and 1.5% of subjects 9 to 17 years. In adults solicited injection site reactions were more frequently reported in Dengue group compared to placebo, whereas similar reports were observed in Dengue and Placebo groups in subjects aged 9-16 years. Headache, malaise, myalgia and asthenia were vey commonly reported in both age groups and with Grade 3 solicited systemic reactions in approximately 10%. 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.

For potential allergic reactions within 7 days, a total 6 cases of rash were reported in adults (6/1547) and 2 cases in subjects aged 9 to 17 years (2/3068). A total of 4 cases of urticarial were reported in children aged 9 to 17 years (4/3068).

Three abnormal pregnancy outcomes were observed in 22 women who were exposed to the CYD dengue vaccine during their pregnancy (death in utero, stillbirth and blighted ovum). In all cases risk factors were identified.

In integrated safety analyses the frequency and nature of SAEs were similar between dengue vaccine and control groups. Within 28 days after any injection, 0.6% of adolescents to 1.0% of adult subjects reported at least one SAE. No cluster of clinical patterns of SAEs up to 28 days post-injection was observed. From 28 days to 6 months after any injection, the proportions of subjects with at least one SAE ranged from 2.4% in adolescents to 3.6% in toddlers. These events were isolated in nature and frequency and were mainly in SOC 'Infections and Infestations'.

In Study CYD14, among SAE, 2 were assessed as treatment related, 1 acute disseminated encephalitis an 8 year old child onset 7 days after first injection in Dengue vaccine group and 1 allergic angioedema onset 18 days after first injection in control group. Four deaths were reported in Active Phase, all not related to vaccination. One death was reported in control group in active phase (acute lymphoblastic leukaemia) and one death in Hospital Phase in control group (encephalitis).

In Study CYD15, A total of 4 SAEs (< 0.1%) were assessed as related to the vaccine by the Investigator and the Sponsor during the Active Phase. In the Dengue vaccine group, acute polyneuropathy, asthma and allergic urticarial were reported and occurred between few hours to 3 days post Injection 1 or Injection 2. In addition, a subject with unspecified seizures onset 18 hours after injection was assessed by sponsor as possibly treatment related. In the control group visual impairment was reported 21 hours post injection 1 which recovered 3 days after onset. During the period from the 6 months follow-up up to the end of the first year of the Hospital Phase, no other SAEs were assessed as related to treatment by investigator. 12 deaths were reported in active phase, 6 in each treatment group, and all reported as not related to vaccination.

There were 5 deaths reported during the first year of the Hospital Phase, 4 in the CYD Dengue Vaccine Group and 1 in the Control Group. None of deaths was assessed as related to product by the Investigator or the sponsor.

In CYD23, a total of 586 SAE were reported. The percentages of SAEs during Active Phase were similar between dengue vaccine and control groups. Only 1 SAE was assessed as treatment related: a subject with acute febrile illness. Five deaths were reported, 4 in control group and 1 in dengue vaccine group. No deaths were considered as related to treatment.

Other safety parameters

SVCD

No evidence of increased risk of SVCD was observed in the Dengue Group compared to the Control Group during the 25-month observation period of active surveillance in each of the 3 efficacy studies overall or in the pooled analysis: 27 SVCD cases were reported in subjects 9 to 16 years who received at least 1 injection in the efficacy studies (4 in the Dengue Group and 23 in the Control Group) with a RR of 0.087 (95% CI: 0.02; 0.25).

Long-term safety follow-up

At the time of submission, the following data from on-going long-term follow-up are available from the efficacy studies:

- Study CYD57: full data from the first 2 years of Hospital Phase (Hospital Phase Year 1 and Year 2, that is, 2 and 3 years after the end of the Active Phase in Study CYD23) and preliminary data from the third a and fourth years of Hospital Phase (cut-off date on 13 August 2015).

- Studies CYD14 and CYD15: full data from the first year of Hospital Phase (Hospital Phase Year 1, that is, 2 years after the end of the Active Phase in Studies CYD14 or CYD15 and 3 years after the last injection) and preliminary data from the second and third years of follow-up (Hospital Phase Year 2 and Year 3) (cut-off date on 13 August 2015).

No SAE safety concerns were identified during long-term follow-up of all these studies.

Hospitalised VCD cases

The incidence of hospitalised VCD cases during the long-term follow-up of the efficacy Studies CYD14, CYD15 and CYD57 was assessed by age group in each individual study (in subjects aged 2 to 5 years, 6 to 11 years and 12 to 14 years), as applicable.

The analyses from the Hospital Phase in CYD14 showed a higher incidence of hospitalised VCD cases in the Dengue Group compared to the Control Group in subjects aged 2 to 5 years at enrolment. The annual incidence rate of hospitalised VCD cases was 1.0% in the dengue group and 0.1% in the placebo group representing a relative risk (RR) of 7.454 (95% CI: 1.15; 313.80). In subjects aged 6 years and above at enrolment, the RR of hospitalised VCD cases was below 1 during the first year of the Hospital Phase, and this RR decreased with increasing age (RR < 1: 0.627 (95% CI: 0.22; 1.83) in subjects aged 6 to 11 years and RR < 1: 0.249 (95% CI: 0.02; 1.74) in subjects aged 12 to 14 years).

In the long-term follow-up of the Phase IIb efficacy Study CYD57 (with subjects aged 4 to 11 years at inclusion in Study CYD23); RR of hospitalised VCD cases varied each year and according to serotypes distribution and age groups. As for Study CYD14, the RR fluctuated over time in young age groups (4 to 5 year-old subjects), with RR of 2.443 and 0.814 the first and second years of hospital phase, respectively, while RR remained consistently below < 1 in older age groups (6 to 11 year-old subjects).

Conversely for Study CYD15, the analyses from the first year of Hospital Phase did not show any difference of incidence of hospitalised VCD cases between the CYD dengue and control groups. The annual incidence rate of hospitalised VCD cases in 9 to 11 year-old subjects was 0.2% in the dengue group and 0.3% in the placebo group representing a relative risk (RR) of 0.554 (95% CI: 0.20; 1.54). Similarly, the annual incidence rate of hospitalised VCD cases in 12 to 16 year-old subjects was < 0.1% in the dengue group and 0.2% in the placebo group representing a relative risk (RR) of 0.501 (95% CI: 0.13; 1.87).

To further inform the benefit/risk ratio of the CYD dengue vaccine, breakdown analyses were performed at different age cut-offs. The cut-off below and above 9 years of age was chosen defining different age groups: (i) subjects aged between 2 and 8 years and (ii) subjects aged between 9 and 14 years). The results in Studies CYD14 and CYD57 were compared to that observed in subjects from 9 years of age included in Study CYD15 where RR was consistently < 1 during the first year of Hospital Phase. In both Studies CYD14 and CYD57, the analysis shows a lower RR in subjects aged 9 to 14 years compared to children aged 2 to 8 years. The RR in subjects aged 9 to 14 years in CYD14 (0.572) was similar to RR in subjects aged 9 to 16 years measured in CYD15 (0.533).

When comparing RR against hospitalised VCD cases in subjects aged 9 to 14 years during the Active Phase in CYD14 and CYD15 (0.185 and 0.197, respectively), to the RR during the first year of Hospital Phase (0.572 and 0.533, respectively), there appears to be a trend toward a higher risk of hospitalised VCD cases in this age group. However, when considering the cumulative data collected during the Active Phase and the first year of Hospital Phase in subjects aged 9 to 14 years included in Studies CYD14 and CYD15, the RR during the entire study remained significantly < 1 with a value of 0.273 (95% CI: 0.14; 0.50) and 0.284 (95% CI: 0.18; 0.44), respectively. These results are in favour of a decreased risk of hospitalised VCD cases in the Dengue Group throughout the studies. The

same trend was observed in Study CYD57, with RR in subjects aged 9 to 11 years significantly < 1 during the entire study (0.290 (95% CI: 0.13; 0.62)).

Hospitalised VCD cases observed during the first year of Hospital Phase in the Dengue Group did not show a different clinical profile in terms of severity compared to the Control Group in the Hospital Phase and also compared to the Active Phase.

Case viraemia levels were similar during the Active and the Hospital Phases.

Safety conclusion

The clinical evaluation report concludes there is no evidence, clinically, immunologically or virologically, that the disease in the Dengue Group is different to that observed with wild-type infection in Control Group. At the time of cut-off date for Hospital Surveillance/Phase data presentation (13 August 2015), preliminary data collected in Studies CYD57, CYD14 and CYD15 during uncompleted years of long term follow-up show the same trend, that is, a favourable benefit/risk ratio in subjects aged 9 years old and above but an increased risk of hospitalised VCD including severe in subjects below 9 years of age.

In subjects aged 2 to 8 years (as seen in Studies CYD14 and CYD23) Hospital Phase available data signal an increase in VCD cases requiring hospitalisation (severe and non-severe), that is, potential risk of increase in severity of dengue disease in this age group.

Benefit-risk assessment

Data from the clinical development of the CYD dengue vaccine has shown that with a 3 dose regimen administered 6 months apart the vaccine is efficacious at the prevention of dengue disease in the subjects aged 9 to 16 years. Efficacy was observed against each of the 4 serotypes with high efficacy seen against severe VCD cases and hospitalised VCD cases over a 25 month observation period. The high post-injection titres seen in adults living in endemic areas in Asia allow us to theoretically bridge immunologically to an adult population.

No evidence of increased severity of dengue disease or increase in frequency of hospitalised Dengue cases has been observed in the 9 to 16 year olds. From this, the benefit/risk balance of the CYD dengue vaccine is positive.

Risk management plan

The clinical evaluator had the following comments regarding the RMP:

> *There is an extensive RMP which seems appropriate. The major issues already identified (specific to the use of this vaccine) are:*
>
> - *The duration of immunity is unknown*
> - *The potential for increasing the severity of virologically confirmed dengue (VCD) in children under 9. This needs further evaluation from follow up study data when available.*

At the second round, the RMP evaluator noted that the sponsor's response to the first round evaluation and had responded to the RMP questions and that there were no new clinical evaluation report issues.

The second round evaluation includes three new and outstanding recommendations:

- (outstanding) The nonclinical evaluation raised the concern that use of Dengvaxia may sensitise individuals to other flaviviruses, such as Zika virus, Murray Valley encephalitis virus, Kunjin virus. Interaction of Dengvaxia with other flaviviruses

should be included as missing information in the summary of safety concerns. The sponsor should propose pharmacovigilance activities to monitor for this theoretical risk. Risk minimisation is not required at this stage.

- The sponsor should address any outstanding issues in the ACSOV advice that have not already been addressed in the sponsor's response.
- The evidence described from the sponsor's literature review indicates the safe use of vaccines in this population more generally. As the RMP clearly states, there is no experience of the use of Dengvaxia in patients with renal or hepatic impairment they should be included as items of Missing Information).

ACSOV meeting 13 advice

The following recommendations should be addressed:

- The committee noted that off-label use in Australia would likely be in non-endemic areas where dengue outbreaks occur, and in these areas important co-morbidities disproportionately affecting the Aboriginal and Torres Strait Islander populations should also be considered. These co-morbidities include diabetes mellitus, hepatitis C (with or without treatment), alcohol and drug abuse, and concomitant vaccines. The committee recommended that these co-morbidities be included under Missing Information in the Summary of Safety Concerns in the ASA.
- The committee advised that the pharmacovigilance plan was inadequate, principally as there is no provision to collect data on the experience of vaccine administration in sero-negative individuals who live in non-endemic regions i.e. the situation in Australia. The committee advised that there is a lack of data on vaccine use in certain age groups in endemic and non-endemic regions, as well as a lack of data on vaccine use in the Australian Aboriginal and Torres Strait Islander populations.
- The committee advised that careful consideration is needed to determine which pharmacovigilance activities are implemented to screen for adverse events in the Australian setting. Active surveillance (such as computer-based automated surveillance tools to send SMSs or web-based surveys to recently vaccinated persons, or extraction of information from software used in general practices and travel medicine clinics) would enhance the pharmacovigilance plan and should be included in the ASA.
- Paediatric off-label administration should be monitored by routine pharmacovigilance and reported in periodic safety update reports with a particular focus on any unexpected safety signals.

Discussion

The Delegate considers the benefit-risk assessment in the clinical evaluation report is appropriate.

The sponsor's cover letter identifies populations in Australia at risk of contracting dengue. The sponsor identified 'at-risk' populations include:

- Residents living in north Queensland where there are annual outbreaks of dengue
- Migrants from endemic countries and their dependents living in Australia who visit their homeland;
- Government departments personnel and their dependent (such as Department of Foreign Affairs and Trade, Australian Defence Force) who are deployed to endemic areas;

- Any person (such as expatriates) intending to reside in endemic countries.

The sponsor cover letter also states that as part of the evaluation process the sponsor will engage with TGA on the indication and specific use of Dengvaxia in intended target populations in Australia.

The clinical evaluation report has commented that no immunological correlate of protection has been established the CYD dengue vaccine. After CYD dengue vaccine, seropostivity rates were high against all dengue serotypes but vaccine efficacy was much lower and varied by serotype. The sponsor in a Pre-submission meeting with TGA argued that analyses of immunogenicity in clinical efficacy trials have established correlates of risk, as follows:

- GMTs higher in non-case subjects than in case subjects for all 4 serotypes and for each treatment group
- Clear association demonstrated between the PD3 titres and the probability of dengue disease for each of the 4 serotypes. The higher the titre, the lower the probability of disease.
- Clear association observed between the PD3 titres and with VE (Fred Hutch analyses) for each of the 4 serotypes: the higher the titre, the higher the VE, with a larger VE amplitude for serotypes 1 and 2.
- However, neutralising Ab titres as measured by the PRNT may predict VE with some variability: some factors like age and/or dengue baseline status seem to interact with neutralising Ab titres for VE prediction, particularly for a low level of titre.

In a meta-analysis pooling results from the Phase III efficacy Studies CYD14 and CYD15, in subjects dengue seronegative at baseline a VE estimate at 52.5% (95% CI: 5.9,76.1) was shown demonstrating a benefit in this population.

The clinical evaluation report accepted bridging from immunogenicity studies conducted in adults aged 18 to 45 years living in endemic areas to immunogenicity and efficacy results in Studies CYD14 and CYD15. The CER also accepted that VE would be expected to be similar for the 46 to 60 years adults living in endemic areas.

The Phase III lot-to-lot consistency Study CYD17 was conducted in Australia but provides a very limited bridge to immunogenicity, efficacy and safety demonstrated in Studies CYD14 and CYD15.

In a 3 dose regimen administered 6 months apart the vaccine is efficacious at the prevention of dengue disease in the subjects aged 9 to 16 years. It was not possible to explore the efficacy of only 1 or 2 injections over a long period of time although efficacy was observed between injections. The 12 month dosage regimen further limits potential for use of this vaccine in Australia.

The efficacy data described is based on 25 month follow-up of the Active Phase of the 2 Phase III efficacy studies (up to 13 months after completion of the 3 dose vaccine regimen). During this time period, efficacy persisted with no evidence of waning. The Sponsor should identify in the Pre-ACV response if additional analyses have become available from these Phase III efficacy studies.

Safety data are available for approximately 28,600 subjects aged 9 months to 60 years who received at least 1 injection of the final formulation. The majority of these subjects are children and adolescents with 1982 adults aged 18 to 60 years of which 241 were over 45 years.

The principal safety concern identified in the clinical development program is a potential for increasing VCD cases requiring hospitalisation in children under 9 years of age.

The sponsor was requested to comment to a second round question:

'[The] TGA has noted a number of publications by Halstead and co-authors and also recently by Ferguson et al., raising concerns about the safety of dengue vaccine including the proposition that 'The combination of poor protection against DENV infection of individuals with circulating DENV antibodies (monotypic immune equivalents) satisfies the known preconditions for antibody-dependent enhancement of infection'.

The sponsor's response is considered reassuring by the Delegate.

The second round RMP evaluation includes 3 new and outstanding recommendations that the sponsor was requested to address:

- (outstanding) The nonclinical evaluation raised the concern that use of Dengvaxia may sensitise individuals to other flaviviruses, such as Zika virus, Murray Valley encephalitis virus, Kunjin virus. Interaction of Dengvaxia with other flaviviruses should be included as missing information in the summary of safety concerns. The Sponsor should propose pharmacovigilance activities to monitor for this theoretical risk. Risk minimisation is not required at this stage.
- The sponsor should address any outstanding issues in the ACSOV advice that have not already been addressed in the post-first round response.
- The evidence described from the sponsor's literature review indicates the safe use of vaccines in this population more generally. As the RMP clearly states there is no experience of the use of Dengvaxia in patients with renal or hepatic impairment they should be included as items of Missing Information.

Request for ACV advice

The committee is requested to provide advice on the following specific issues:

1. The ACV is asked for advice on the Indications appropriate for registration of Dengvaxia in Australia. The global Core Company Datasheet indications wording reflects available efficacy and safety experience. Australia is a reference country for therapeutic products marketing authorisation for some countries in the Asia-Pacific region, including some endemic countries. The ACV may comment on whether indications should identify Australian populations at risk of contracting dengue.
2. The benefit-risk balance of Dengvaxia in the ongoing efficacy and safety, Studies CYD14, CYD15 and CYD 57, including the adequacy of the sponsor response on the publications raising concern over safety of this vaccine in relation to antibody dependant enhancement of infection.
3. The second round RMP evaluation includes 3 new and outstanding recommendations that the sponsor was requested to address.

The committee is also requested to provide advice on any other issues that it thinks may be relevant to a decision on whether or not to approve this application.

Proposed action

The Delegate has no reason to say, at this time, that the application for dengue tetravalent vaccine (live, attenuated), Dengvaxia, should not be approved for registration, subject to ACV advice and sponsor response to new RMP evaluation recommendations.

Response from sponsor

The sponsor's comments on the issues for which the advice of the ACV is sought, as outlined in the Delegate's Overview, are presented below.

1. ***The ACV is asked for advice on the Indications appropriate for registration of Dengvaxia in Australia. The global Core Company Datasheet indications wording reflects available efficacy and safety experience. Australia is a reference country for therapeutic products marketing authorisation for some countries in the Asia-Pacific region, including some endemic countries. The ACV may comment on whether indications should identify Australian populations at risk of contracting dengue.***

As noted by the Delegate and discussed during the Pre-submission Meeting, the sponsor submitted the following indication, which is reflective of the clinical development program being conducted primarily in endemic countries; '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'.

2. ***The benefit-risk balance of Dengvaxia in the ongoing efficacy and safety Studies CYD14, CYD15 and CYD 57, including the adequacy of the Sponsor response on the publications raising concern over safety of this vaccine in relation to antibody dependant enhancement of infection.***

No new information became available since our initial response so our conclusions stated in the response document remain unchanged, which are:

- Overall, vaccination in endemic areas is expected to provide long term benefit, whichever serostatus;
- Clinical data in seronegative individuals from efficacy studies (CYD23/47, CYD14 and CYD15) demonstrate that there are no safety concerns reported to date in seronegative subjects aged 9 years and above, vaccinated with the dengue vaccine.

3. ***The second round RMP evaluation includes 3 new and outstanding recommendations that the sponsor was requested to address.***

The sponsor's responses to the new and outstanding recommendations are provided in the response to the RMP.

In addition, the sponsor is providing the following information to address the Delegate's requests specified in the Delegate's Overview.

1. ***The efficacy data described is based on 25 month follow-up of the Active Phase of the 2 Phase III efficacy studies (up to 13 months after completion of the 3-dose vaccine regimen). During this time period, efficacy persisted with no evidence of waning. The Sponsor should identify in the Pre-ACV response if additional analyses have become available from these Phase III efficacy studies.***

Efficacy data have been collected up to 25 months after the first injection. Long-term efficacy data are currently being collected in CYD14 and CYD15 surveillance expansion phase. Data from the surveillance expansion phase will be available after completion of the studies and will provide further information on the long-term protection of the vaccine.

2. ***The principal safety concern identified in the clinical development program, as seen in Studies CYD14 and CYD23, is an increase in VCD cases requiring hospitalisation in children under 9 years of age. The sponsor should identify in the Pre-ACV response if additional analyses have become available from the efficacy studies, beyond those included in the 28 March 2017 responses to Clinical Question (at the) second round.***

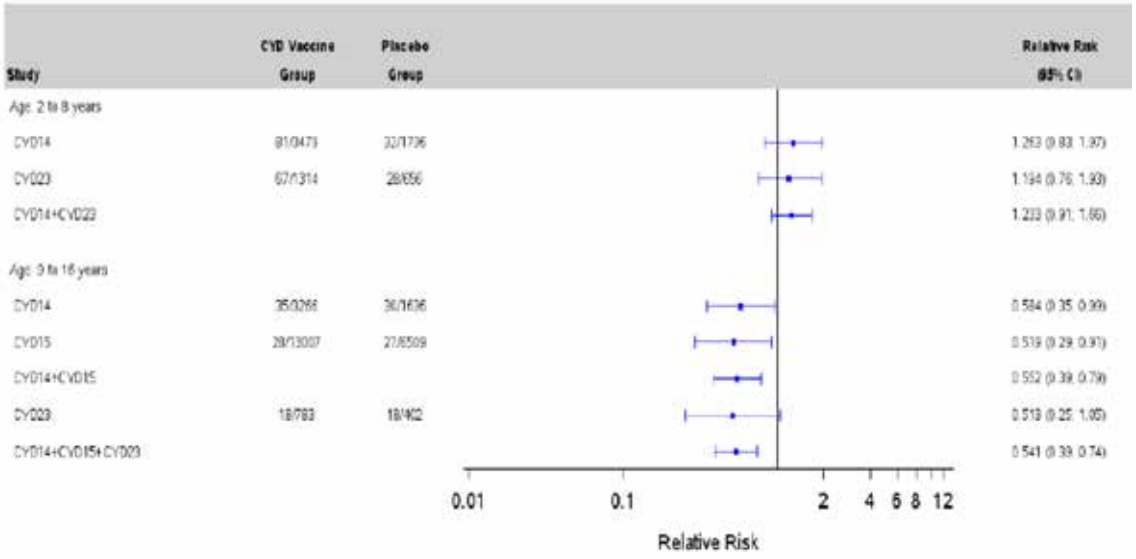
Data from hospitalised and/or severe virologically confirmed dengue (VCD) are now available up to a latest cut-off on 11 November 2016 for CYD15 and 5 December 2016 for Study CYD14. In the two Phase III studies all VCD cases are being collected following the protocol amendments 4. Data collected since the previous cut-off on uncompleted year are

considered as preliminary at this point as they are extracted from unlocked databases of uncompleted follow-up.

Additional data from the ongoing long-term follow-up (LTFU) indicate that the overall benefit-risk evaluation remains positive in the indicated population, i.e. in children 9-16 years of age as confirmed by the IDMC (Independent Data Monitoring Committee; cumulative relative risk (RR) on hospitalised dengue during LTFU = 0.37 (95% CI 0.30 to 0,47)) (see Figure 7).

Outside the approved indication, in the younger age groups in children 2 to 8 years of age from Studies CYD14 and CYD23/57, the imbalance on hospitalised and/or severe cases appears to have stabilised (cumulative RR on hospitalised dengue during LTFU = 0.9 (95% CI 0.73 to 1.12)) (see Figure 7).

Figure 7: Forrest plot for relative risk against hospitalised and/or severe (IDMC) VCD cases due to any of the 4 serotypes during the Hospital Phase/Surveillance Expansion Phase (SEP), by age groups (Safety Analysis Set, up to February/March 2016 cut-off)



The numerator is the number of subjects with a hospitalised and/or severe (IDMC) virologically-confirmed dengue episodes in the considered period. Integrated Relative Risk and Confidence Intervals are calculated using Cox regression model. Relative Risk of a study is calculated using Density incidence: cases per 100 persons The denominator is the mean of number of subjects followed during the completed years included in the Hospital Phase /SEP.

The imbalance on hospitalised and/or severe cases is mainly driven by 2 to 5 year-old subjects (RR on hospitalised dengue = 1.786 (95% CI 1.03 to 3.26) during the LTFU in Study CYD14), in which the RR was higher than in 6-8 year-old subjects (RR on hospitalised dengue = 0.784 (95% CI 0.48 to 1.31) during the LTFU in Study CYD14).

As previously observed, there was no difference in the clinical picture of severity, with comparable frequencies of various signs and symptoms of dengue severity, length of hospitalisation and duration of fever in the Hospital Phase (HP) between vaccine and control groups, and between HP and Active Phase.

As regards to the occurrence of hospitalised VCD in subjects who were seronegative at baseline, data collected up to the latest cut-off show that there are no safety concerns reported to date in seronegative subjects aged 9 years and above, vaccinated with the dengue vaccine.

New analyses to assess the risk of hospitalised dengue in seronegative subjects from 9 years and above is presented below and include additional complete Y3 HP data of Study

CYD14, incomplete Y3 HP data of Study CYD15, and incomplete Y4 HP data of Studies CYD14 and CYD15 with a cut-off date of 5 December 2016 and of 11 November 2016 respectively, and the totality of the LTFU of Study CYD57.

- While these data are based on a limited sample size of subjects in the immunogenicity subset who are seronegative, they do not demonstrate a significantly increased risk of hospitalised dengue in the indicated seronegative population (Table 12).
- No clinical shock was reported in dengue seronegative subjects, according to WHO definition.
- There was no difference in the clinical severity of hospitalised dengue cases between cases in subjects who were seronegative at baseline between the vaccinated and control groups, although the overall low number of severe cases makes the interpretation difficult.

Table 12: Hospitalised VCD cases during the LTFU and Overall in Seronegative subjects above 9 years of age at Inclusion (up to November/December 2016 cut-off)

			Dengue Group (N=23433)				Control Group (N=11690)			
Age Group	Study	Time Period	Cases	M	Annual Incidence Rate (95% CI)	n Episodes	Cases	M	Annual Incidence Rate (95% CI)	n Episodes
9-16 years	CYD57	Y1-Y4 HP	1	12	8.3 (0.2; 38.5)	1	0	8	0.0 (0.0; 36.9)	0
		Entire Study	1	13	1.3 (0.0; 7.0)	1	0	8	0.0 (0.0; 7.3)	0
	CYD14	Y1-Y4 HP/SEP	5	124	1.4 (0.5; 3.2)	5	4	58	2.4 (0.6; 5.9)	4
		Entire Study	5	126	0.8 (0.3; 1.8)	5	5	58	1.7 (0.6; 4.0)	6
	CYD15	Y1-Y4 HP/SEP	3	239	0.4 (0.1; 1.3)	3	0	134	0.0 (0.0; 0.9)	0
		Entire Study	3	245	0.2 (0.1; 0.7)	3	0	139	0.0 (0.0; 0.5)	0

M: number of subjects present at the beginning of each year or mean of number of subjects followed during the completed years included in the considered period, except for the Hospital Phase and the Surveillance Expansion Phase for which the denominator (M) will be the person-years followed in the two phases. Cases: number of subjects with at least one hospitalised and/or severe (IDMC) virologically-confirmed dengue episode in the considered period n Episodes: number of hospitalised and/or severe (IDMC) virologically-confirmed dengue episodes in the considered period Annual Incidence rate= Cases among M * 100 converted in annual rate. Confidence Intervals for the single proportion are calculated using the exact binomial method (Clopper-Pearson method; quoted by Newcomb).

Advisory Committee Considerations[62]

The Advisory Committee on Vaccines (ACV), taking into account the submitted evidence of efficacy, safety and quality, agreed with the Delegate and considered Dengvaxia dengue tetravalent vaccine (live, attenuated) to have an overall positive benefit-risk profile for the amended indication (changes recommended by the committee in **bold**):

> *Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through **45 years** of age living in endemic areas **where prevalence of past infection exceeds 50%. Use should be in accordance with official guidelines.***

In making this recommendation, ACV:

- considered that the vaccine is moderately effective with no major safety concerns, except in age groups not proposed for approval;

[62] ACV provides independent medical and scientific advice to the Minister for Health and TGA on issues relating to the safety, quality and efficacy of medicines supplied in Australia, including issues relating to pre- and post-market functions for medicines. ACV is established under Regulation 35 of the *Therapeutic Goods Regulations 1990*. Members are appointed by the Minister. Membership comprises of professionals with specific scientific, medical or clinical expertise, as well as appropriate consumer health issues relating to medicines.

- noted that dengue fever is not currently endemic in any geographic area or population in Australia, although this could change in the future;
- noted that clinical studies of efficacy and safety have all been performed in highly endemic settings with poor and unknown generalisability to Australian sub-populations who may be interested in seeking dengue immunisation (that is, travellers, migrants, expatriates, residents of far north Queensland); and
- noted the lack of safety data in some sub-populations (for example, persons with multiple co-morbidities).

Proposed PI/CMI amendments

The committee emphasised that the PI and CMI should provide sufficient information to allow prescribers and consumers to make a determination of the risks and benefits of the vaccine. The key concerns to be communicated should be:

- the vaccine is not indicated for people living in non-endemic areas
- the vaccine is not indicated for those travelling to endemic areas
- the vaccine is contraindicated in children under 9 years of age because of a safety signal indicating that there is a higher risk of hospitalisation due to severe dengue following exposure to wild-type virus
- the potential risk of sensitisation and development of severe dengue disease in sero-negative persons who are vaccinated and then contract the wild-type mosquito-borne virus; this should be a key issue for those intending to travel and reside for long periods of time in endemic areas. Such individuals should consider the determination of serology prior to vaccination and should only be vaccinated if sero-positive. However, safety and efficacy has not been evaluated under these circumstances
- there is reduced efficacy in persons who are sero-negative prior to immunisation
- there is a lack of long term efficacy data and the need for booster doses is unknown.

ACV advised that consideration should be given to including renal impairment and hepatic impairment as contraindications, until further data become available.

Specific advice

ACV advised the following in response to the Delegate's specific questions on the submission:

1. ***The ACV is asked for advice on the Indications appropriate for registration of Dengvaxia in Australia. The global Core Company Datasheet indications wording reflects available efficacy and safety experience. Australia is a reference country for therapeutic products marketing authorisation for some countries in the Asia-Pacific region, including some endemic countries. The ACV may comment on whether indications should identify Australian populations at risk of contracting dengue.***

ACV advised amendments to the proposed indication regarding age group, 'living in endemic areas' and relevant clinical practice.

ACV noted that the clinical studies were all performed in highly endemic settings with poor generalisability to Australian populations.

Further data are required in populations at risk, for example, travellers, before the vaccine could be considered for use outside of endemic areas.

Age group of 9 to 45 years

The committee advised that safety data in persons aged 46 to 60 years (n = 241) were lacking, with no data on immunogenicity or protection. All but one country where Dengvaxia is already approved have imposed 45 years as the upper age limit.

'Living in endemic areas'

The committee noted the international position from the WHO's Strategic Advisory Group of Experts (SAGE) on Immunisation is that populations with high rates of dengue seropositivity (above 70%) should be targeted for vaccination, to both maximise vaccine performance and to minimise any theoretical risks of vaccination, and that the vaccine is not recommended when seroprevalence is below 50% in the age group targeted for vaccination. The seropositivity of dengue in blood donors in Queensland is currently about 10%. The committee concluded that there is currently no population in Australia in which dengue is considered to be endemic and so suited to vaccination. The inclusion in the indication of **where prevalence of past infection exceeds 50%** is consistent with the WHO position.

It was not appropriate to identify Australian populations 'at risk of contracting dengue', but not living in endemic areas, in the indication. There were no bridging studies from the clinical studies in endemic areas to non-endemic areas.

'Use in accordance with official guidelines'

The committee viewed this caveat as reasonable for this vaccine and where Australia may be considered as a reference country for the purposes of other national regulatory bodies in the Asia-Pacific region.

2. The benefit-risk balance of Dengvaxia in the ongoing efficacy and safety CYD14, CYD15 and CYD 57, including the adequacy of the Sponsor response on the publications raising concern over safety of this vaccine in relation to antibody dependant enhancement of infection.

The committee advised that the risk-balance was positive in the populations studied, excluding children under 9 years of age. Thus, the vaccine is moderately effective with no major safety concerns, except in age groups not proposed for approval.

There were few significant local reactions to the vaccine, but clinically significant systemic reactions did occur in about 10% of subjects. No cases of anaphylaxis were recorded. No fatality was considered related to vaccination (in total 12 deaths occurred in the vaccinated groups and 6 in the control groups). Serious adverse events following immunisation included acute disseminated encephalomyelitis (7 days after dose 1), angioedema (18 days), acute polyneuropathy and asthma. The committee was concerned that excess hospitalisations seen in children under 9 years of age may reflect immunological enhancement in unexposed individuals.

The committee noted the difference in views between the sponsor and Halstead and other authors. Resolution of the current controversy awaits further data.

3. The second round RMP evaluation included three new and outstanding recommendations that the sponsor was requested to address.

a. the concern that use of Dengvaxia may sensitise individuals to other flaviviruses, such as Zika virus, Murray Valley encephalitis virus, Kunjin virus. Interaction of Dengvaxia with other flaviviruses should be included as Missing Information in the Summary of Safety Concerns.

The committee advised that the sponsor's position was acceptable, to not include Interaction of Dengvaxia with other flaviviruses as Missing Information in the RMP. The

risk of sensitisation appeared to be theoretical and monitoring of dengue and Zika interactions in Study CYD15 (from 2013 onwards) will provide information in due course.

b. outstanding issues in the ACSOV 13 advice

i. co-morbidities disproportionately affecting the Aboriginal and Torres Strait Islander populations, and concomitant vaccines, should be considered as Missing Information in the Summary of Safety Concerns

The committee did not agree with the sponsor's response that immunogenicity and tolerance of the vaccine in individuals with co-morbidities had been demonstrated. The committee noted that co-administration of the vaccine with human papillomavirus vaccine or booster tetanus/diphtheria/acellular pertussis vaccine has been included as Missing information in version 2.0 of EU RMP.

ii. adequacy of the pharmacovigilance plan, which has no provision to collect data on the experience of vaccine administration in sero-negative individuals who live in non-endemic regions, that is, the situation in Australia.

The committee did not agree with the sponsor's response. Administration of the vaccine to persons residing in Australia will be 'off-label' and the proposed pharmacovigilance plan will not necessarily provide information on the safety of use of the vaccine in Australia. The amended wording of the indication should assist in reducing off-label use.

iii. active surveillance (for example, computer-based automated surveillance tools to send SMSs or web-based surveys to recently vaccinated persons, or extraction of information from software used in general practices and travel medicine clinics) to enhance the pharmacovigilance plan

The committee considered the sponsor's response to 'consider implementing' active surveillance as insufficient for the vaccine if/when marketed in Australia.

Monitoring for disseminated dengue vaccine disease should be required.

iv. paediatric off-label administration

The committee found the sponsor's response to be inadequate, and as above, active surveillance should be undertaken. Measures to mitigate the potential immunisation error of administration to children under 9 years should be considered. A checklist approach may not be sufficient and consideration should be given to inclusion of the indicated age group on the vaccine labelling.

c. use of Dengvaxia in people with renal or hepatic impairment should be included as items of Missing Information in the Summary of Safety Concerns

The committee found the sponsor's response to be inadequate. Renal and hepatic impairments pose both safety and efficacy concerns: the safety issue is the potential for disseminated vaccine infection and the efficacy concern is the potential for poor immunogenicity.

Outcome

Based on a review of quality, safety and efficacy, TGA approved the registration of Dengvaxia dengue tetravalent vaccine (live, attenuated), powder and diluent for suspension for injection indicated for:

> *the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age living in endemic areas. Use should be in accordance with official guidelines (see 'Dosage and Administration').*

Specific conditions of registration applying to these goods

RMP

- The Dengvaxia RMP: EU-RMP (version 2.0, dated10 January 2017, data lock point 18 September 2016) with ASA (version 1.2, dated 30 June 2017), and any future updates, as agreed with the TGA will be implemented in Australia.

Batch Release Testing and Compliance with the Certified Product Details

- It is a condition of registration that all independent batches of Dengvaxia Dengue tetravalent vaccine (live, attenuated) imported into Australia are not released for sale until samples and the manufacturer's release data have been assessed and you have received notification from the Laboratories Branch, TGA, that there is no objection to you releasing the product to the Australian market.
- For each independent batch of the product imported into Australia, the sponsor must supply the following:
  - A completed Request for Release Form
  - Complete summary protocols for manufacture and QC, including all steps in production.
  - At least 5 doses of each first consignment of product lot with the Australian approved labels, PI and packaging. 3 doses of any further consignment of already released product (including diluents) with the Australian approved labels, PI and packaging.
  - Certificate of Release from a regulatory agency acting for the country of origin such as an OMCL (if available).
  - Any reagents, reference material and standards required to undertake testing, as requested by Laboratories Branch, TGA.

Post outcome

Dengvaxia was registered by TGA in July 2017. At the time of preparing this AusPAR, Dengvaxia had not yet been marketed in Australia, although it had been approved in a total of 16 dengue endemic countries including Mexico, The Philippines,[63] Brazil, El Salvador, Costa Rica, Guatemala, Indonesia, Peru, Bolivia, Singapore, Cambodia, Thailand, Paraguay, Venezuela, Argentina and Malaysia.

Following the initial registration, the sponsor applied to TGA with the following:

- Safety Related Request (SRR), with data and minor editorial change;
- Amendments to the PI, which included changed dosage information. The currently approved dosage information is:

  The primary vaccination schedule consists of 3 injections of 0.5 mL to be administered by subcutaneous injection at 6 month intervals.

  If flexibility in the vaccination schedule is necessary, a time window of +/- 20 days is acceptable.

  The vaccine must be used according to official guidelines: in the context of a vaccination campaign, the vaccine is recommended in endemic areas where the

[63] After being granted market authorisation in The Philippines on 22 December 2015, the licence was subsequently suspended until 2 January 2019.

seroprevalence is above 50% or in areas where epidemiological data indicate a high burden of disease.

The proposed dosage information amended to third paragraph to:

The vaccine must be used according to official guidelines. For countries considering vaccination as part of their dengue control program, WHO have recommended a 'pre-vaccination screening strategy' as the preferred option, in which only dengue-seropositive individuals are vaccinated (see Section 4.4 Special warning and precautions for use).

Delegate's summary of issues

The clinical program of the CYD dengue vaccine included two pivotal Phase III efficacy studies (CYD14 and CYD15) that had long-term following continuing at the time of registration and a proof of concept Phase IIb efficacy study (CYD23/57) that was completed at the time of registration.

Results from the Phase III efficacy studies (CYD14 in Asia and CYD15 in Latin America) also showed that vaccine efficacy (VE) was impacted by prior exposure to wild-type dengue infection. In the "immunogenicity subset" for whom serostatus was evaluated at baseline, pooled VE against Virologically-confirmed dengue (VCD) cases in subjects aged ≥ 9 years at enrolment across these 2 pivotal efficacy studies was 81.9% (95% CI: 67.2; 90.0) among subjects ≥ 9 years of age classified as seropositive at baseline compared to 52.5% (95% CI: 5.9; 76.1) among seronegative subjects.

During the first year of the Hospital Phase of CYD14 (i.e. during the third year after the first injection), the Phase III efficacy trial conducted in Asia, there was an imbalance and a significant increased risk of hospitalised and/or severe symptomatic VCD in the youngest vaccine recipients (subjects aged 2 to 5 years at enrolment).

In order to further evaluate the safety and efficacy of the CYD dengue vaccine according to dengue serostatus prior to vaccination, the sponsor has conducted a supplemental exploratory analyses using blood samples for all study participants in the Phase III studies collected at month 13, 1 month after 3rd injection of CYD dengue vaccine.

In November 2017, the sponsor announced the results of the new analyses had shown an increased risk of hospitalised dengue and seronegative dengue in seronegative individuals from year 3 onwards during the 66 month observation period. In subjects 9 years of age and older with no previous dengue infection, it was estimated during 5 years of follow-up approximately 5 additional hospitalised dengue cases and 2 additional severe dengue cases per 1000 vaccinees could occur.

In Australia, the sponsor submitted a safety related request to amend the PI in December 2017.

Section 9D (2) of the *Therapeutic Goods Act 1989* states that 'If:

(a) the person in relation to whom the therapeutic good are registered or listed has requested the Secretary to vary the information included in the entry in the register that relates to the goods; and

(b) the only effect of the variation would be:

(i) to reduce the class of persons for whom the goods are suitable; or

(ii) to add a warning, or precaution, that does not include any comparison of the goods with any other therapeutic goods by reference to quality, safety and efficacy';

the secretary must vary the entry in accordance with the request.'

The supporting data submitted by the sponsor did not initially include study reports and these were requested by TGA. A safety request has a short statutory time frame for finalisation but for this application substantial mutual stop-clocks have been agreed with the sponsor. WHO SAGE announced a review of recommendations on use of dengue vaccine in late 2017. As Dengvaxia is not marketed in Australia and is indicated for individuals living in endemic areas, the review of the application was deferred until the WHO SAGE revised recommendations were available.

The change(s) to the PI proposed by the sponsor can be classified as either a reduction in the class of persons for whom the goods are suitable or a warning or precaution, and therefore these amendments are largely acceptable under section 9D (2) of the Act. However, the clinical evaluator has recommended some amendments and additional statements to the draft PI, particularly to align with WHO SAGE revised recommendations to which the sponsor responded in July 2018.

Request for ACV advice

The committee is requested to provide advice on the following specific issues:

1. Do the proposed PI amendments for dengue tetravalent vaccine (live, attenuated) Dengvaxia adequately reflect the revised safety profile?
2. Is amendment of the 'Therapeutic Indications' section of the PI appropriate? The 'Therapeutic Indications' section currently includes a cross-reference to 'Dose and Method of Administration' in which some relevant statements are included.

The committee is (also) requested to provide advice on any other issues that it thinks may be relevant to a decision on whether or not to approve this application.

Advisory Committee Considerations

ACV advised the following in response to the specific questions from the Delegate of the Secretary of Health.

1. Do the proposed PI amendments for dengue tetravalent vaccine (live, attenuated) Dengvaxia adequately reflect the revised safety profile?

ACV advised that the revised safety profile, outlined above, shows that that there is clear evidence of harm in vaccination of seronegative persons, while the risk-benefit remains favourable for seropositive persons.

ACV advised that the wording of the Therapeutic Indications should be amended to state that the individual must be demonstrated to be seropositive to dengue (see Question 2).

ACV advised that the PI statement on 'travellers' should be revised to complement an amended Indication. Only persons who intend to reside in high dengue prevalence areas should be considered for vaccination. With the primary vaccination schedule requiring 12 months to complete, incomplete administration of the recommended dosing schedules was an identified issue.

The PI heading 'special patient groups' seemed incongruous, as **travellers** and **individuals who have not been previously infected by dengue virus or for whom this information is unknown** could potentially form the majority of persons presenting for vaccination if the vaccine were to be supplied in Australia.

ACV advised that it supported all other PI changes as proposed by the Delegate.

2. Is amendment of the 'Therapeutic Indications' section of the PI appropriate? The 'Therapeutic Indications' section currently includes a cross-reference to 'Dose and Method of Administration' in which some relevant statements are included

ACV advised that it is appropriate to amend the Therapeutic Indications. Suitable wording is:

> *Dengvaxia is indicated for the prevention of subsequent dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age with laboratory-confirmed past dengue infection who are or will be resident in endemic areas.*
>
> *Use should be in accordance with official guidelines. If considered as part of a control program for endemic dengue, WHO have recommended a 'pre-vaccination screening strategy' as the preferred option, in which only dengue-seropositive individuals are vaccinated.*

The revised safety profile shows that potential vaccine recipients should have proven previous dengue infection, in addition to the age and place of residence criteria currently stated in the Indications. The Indication should clearly advise that administration of the vaccine in Australia should only be to persons with documented laboratory-confirmed prior dengue infection. Undocumented medical history is insufficient for making an informed decision. Dengue-like illness during previous residence in a dengue endemic region, in the absence of any laboratory confirmation of infection, is insufficient for making an informed decision.

The cross-reference from the Indication to Dosage section of the PI is insufficient to define the population for which the vaccine is indicated. Screening should be incorporated in the Indications section of the PI as the persons the vaccine is indicated for are persons who are seropositive. Screening relates to patient selection rather than dosage.

Other advice

ACV supported the development of a highly sensitive and specific rapid diagnostic test to determine serostatus. Until that time, usual clinical practices regarding the selection of screening and diagnostic tests would apply, but for screening purposes clinicians should be instructed to use a serologic test with high specificity for dengue virus infection, given the known cross reactivity of some dengue serologic tests with other flaviviruses.

ACV noted the sponsor's undertaking to update the CMI once the application is approved. The committee advised that statements in the CMI such as 'if you do not know whether you or your child has ever been infected by dengue virus' need to be amended, as there should be clear communication that consumers are required to have objective evidence on seropositivity prior to vaccination. The decision to vaccinate needs to be based on documented (not merely recalled) laboratory testing. The likely need for a blood test prior to the vaccine should be mentioned in the CMI.

Post outcome approval

Based on the review of quality, safety and efficacy, TGA approved the registration of Dengvaxia dengue tetravalent vaccine (live, attenuated), powder and diluent for suspension for injection indicated for:

> *Dengvaxia is indicated for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals 9 through 45 years of age with previous dengue infection and living in endemic areas.*
>
> *Use should be in accordance with official guidelines. Previous dengue infection must be demonstrated by history of laboratory-confirmed dengue infection or serotesting according to local official recommendations.*

Attachment 1. Product Information

The PI for Dengvaxia approved with the submission (post outcome) which is described in this AusPAR is at Attachment 1. For the most recent PI, please refer to the TGA website at <https://www.tga.gov.au/product-information-pi>.

Attachment 2. Extract from the Clinical Evaluation Report

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