PUBLIC ASSESSMENT REPORT
FOR
DENGVAXIA, DANGVAXIA MD

Common Name: Dengue tetravalent vaccine (live, attenuated)

Procedure No. 2C 90003/58 (NB), 2C 90004/58 (NB)

Assessment Report as adopted by the TFDA with
all information of a commercially confidential nature deleted

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Sanofi Pasteur Ltd submitted on 9 July 2015 an application for Marketing Authorization to the Thailand Food and Drug Administration (TFDA). At the time of submission and validation, DENGVAXIA and DENGVAXIA MD (multidose) were designated as medicinal product in the following 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.

The legal basis for this application refers to: Drug Act 2510 B.E.

The application submitted was a complete dossier: composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and bibliographic literature substituting/supporting certain tests or studies.

Licensing status:

The product was not licensed in any country at the time of submission of the application.

1.2 Steps taken for the assessment of the product

- The application was received by the TFDA on 29 May 2015
- The procedure started on 9 July 2015

2. SCIENTIFIC DISCUSSION

2.1 Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. An estimated 390 million dengue infections with 96 million clinical cases occur [1]annually and approximately 3.9 billion people live in areas with risk of infection with dengue virus[1].The 2002 World Health Assembly resolution WHA55.17 urged greater commitment to dengue by WHO and its Member States. Of particular significance is the 2005 World Health Assembly resolution WHA58.3 on the revision of the International Health Regulations (IHR) (3), which includes dengue as an example of a disease that may constitute a public health emergency of international concern with implications for health security due to disruption and rapid epidemic spread beyond national borders. WHO has repeatedly updated their fact sheet on dengue and severe dengue (most recently in July 2016)[1], and also has published their first position paper [2]on dengue vaccine in July 2016. WHO recommends that countries should consider the use of the dengue vaccine in geographic settings (national or subnational) where epidemiological data indicate a high burden of disease in parallel to other preventive measures such as vector control.

Dengue virus (DEN) is a small single-stranded RNA virus comprising four distinct serotypes (DEN-1 to -4). These closely related serotypes of the dengue virus belong to the genus Flavivirus, family Flaviviridae. The mature particle of the dengue virus is spherical with a

diameter of 50nm containing multiple copies of the three structural proteins, a host-derived membrane bilayer and a single copy of a positive-sense, single-stranded RNA genome. The genome is cleaved by host and viral proteases in three structural proteins (capsid, C, prM, the precursor of membrane, M, protein and envelope, E) and seven nonstructural proteins (NS). Distinct genotypes or lineages (viruses highly related in nucleotide sequence) have been identified within each serotype, highlighting the extensive genetic variability of the dengue wild-type serotypes. Purifying selection appears to be a dominant theme in dengue viral evolution, however, such that only viruses that are “fit” for both human and vector are maintained. Among them, “Asian” genotypes of DEN-2 and DEN-3 are frequently associated with severe disease accompanying secondary dengue infections. Intra-host viral diversity (quasispecies) has also been described in human hosts.

There was no vaccine approved by Regulatory Authorities at the time of submission of the application.

Clinical Signs and Symptoms

Dengue Fever

The disease manifests as a sudden onset of severe headache, chills, pain upon moving the eyes, and low backache. Painful aching in the legs and joints (myalgias and arthralgias—severe pain that gives it the nick-name break-bone fever or bonecrusher disease) occurs during the first hours of illness. The temperature rises quickly as high as 40° C, with relative low heart rate (bradycardia) and low blood pressure (hypotension). The dengue rash is characteristically bright red petechiae and usually appears first on the lower limbs and the chest (see figure 2). The glands (lymph nodes) in the neck and groin are often swollen. In some patients, it spreads to cover most of the body. There may also be gastritis with some combination of associated abdominal pain, nausea, vomiting, or diarrhea. Some cases develop much milder symptoms which can be misdiagnosed as influenza, chikungunya, or other viral infection when no rash is present. The classic dengue fever lasts about six to seven days, with a smaller peak of fever at the trailing end of the disease (the so-called biphasic pattern). Clinically, the platelet count will drop until the patient's temperature is normal.

Recognition of Dengue fever

- Sudden onset of high fever
- Severe headache (mostly in the forehead)
- Pain behind the eyes which worsens with eye movement
- Body aches and joint pains
- Nausea or vomiting

Dengue virus infections are sometimes confused with chikungunya viral infection, because both diseases can present with high temperatures and myalgias (muscle pain) in people living in or returning from tropical areas (see figure 2, and also diagnosis of Dengue and Chikungunya). Although these diseases share similar clinical features, prominent and prolonged joint pains are more consistent with chikungunya, whereas haemorrhage is more common in cases of dengue virus infection.

Dengue Haemorrhagic Fever

Dengue hemorrhagic fever (DHF) is caused by the same viruses and is characterized by increased vascular permeability, hypovolaemia and abnormal blood clotting mechanisms. DHF is a potentially deadly complication with symptoms similar to those of dengue fever, but after several days the patient becomes irritable, restless, and sweaty. The illness often begins with a sudden rise in temperature accompanied by facial flush and other flu-like symptoms. The fever usually continues for two to seven days and can be as high as 41°C, possibly with convulsions and other complications.

In moderate DHF cases, all signs and symptoms abate after the fever subsides. In severe cases, the patient's condition may suddenly deteriorate after a few days of fever; the temperature drops, followed by signs of circulatory failure, and the patient may rapidly go into a critical state of shock. The Dengue Shock Syndrome (DSS) is characterized by bleeding that may appear as tiny spots of blood on the skin (petechiae) and larger patches of blood under the skin (ecchymoses). Minor injuries may cause bleeding (see figure 4). Shock may cause death within 12 to 24 hours. Patients can recover following appropriate medical treatment.

The progress towards DHF or DSS occur after 3-5 days of fever (see figure 3). At this time, fever has often come down. This may mislead many of us to believe that the patient is heading towards recovery. In fact, this is the most dangerous period that requires high vigilance from care-givers.

Recognition of Dengue Haemorrhagic Fever (DHF)

- Symptoms similar to dengue fever plus, any one of the following:
- Severe and continuous pain in abdomen
- Bleeding from the nose, mouth and gums or skin bruising
- Frequent vomiting with or without blood
- Black stools, like coal tar
- Excessive thirst (dry mouth)
- Pale, cold skin
- Restlessness, or sleepiness

Dengue shock syndrome is defined as dengue hemorrhagic fever plus:

- Weak rapid pulse
- Narrow pulse pressure (less than 20 mm Hg)
- Cold, clammy skin and restlessness.

2.2 Quality aspects

Introduction

Dengue Vaccines

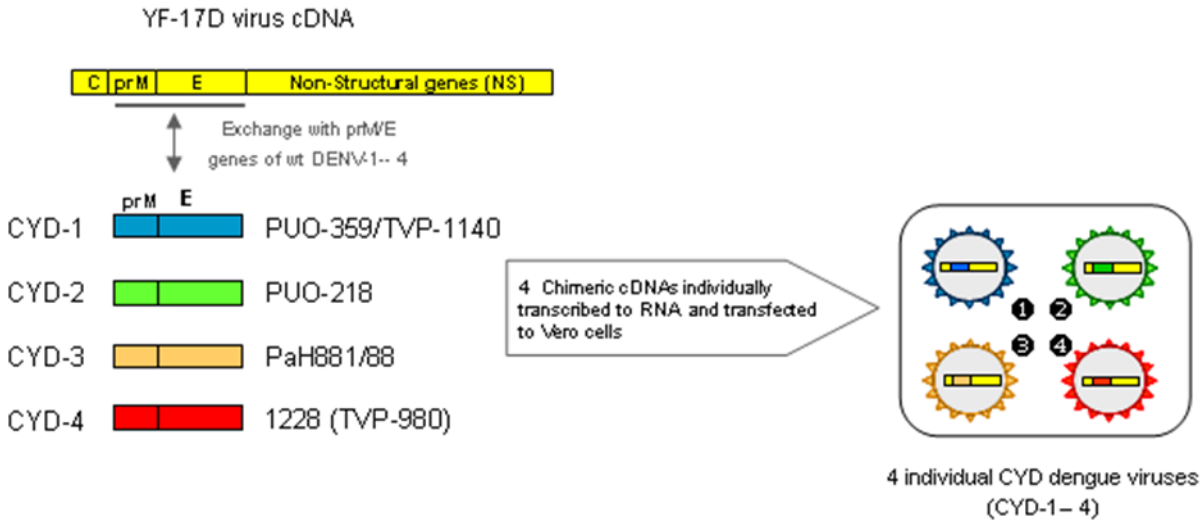


Figure ~~2~~ 1.

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 to 4 via recombinant DNA technology. The CYD dengue viruses were constructed by replacing the sequence encoding the pre membrane (prM) and envelope (E) proteins in the parental yellow fever 17D (YF 17D) virus genome by those encoding 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). This results in the production of 4 CYD dengue virions (one for each serotype), expressing the envelope protein of each wt dengue virus strain at their surface as shown in Figure 2.

It is important to note that the CYD dengue viruses 1 to 4 do not contain genetic information for the prM and E proteins of the YF 17D virus as these sequences have been replaced by those of the corresponding wt dengue viruses.

The manufacture of CYD dengue Drug Substances is divided into 5 major manufacturing process stages:

- Cell culture: The purpose is to amplify the Vero cells, obtained from the WCB.
- Viral culture and clarification: The purpose is to infect the Vero cells with one single serotype of CYD dengue virus seed lot (CYD-1, CYD-2, CYD-3 or CYD-4) to obtain the Crude Harvest which is further filtered to produce the Clarified Harvest;
- Purification: The purpose is to purify the Clarified Harvest by eliminating residual nucleic acid (DNA) from Vero cell substrate;

- Concentration and diafiltration: The purpose is to concentrate and diafiltrate the CYD dengue virus in an optimal salt concentration and pH matrix to obtain the Concentrated Harvest;
- Stabilization, filling and storage: The purpose is to stabilize the Concentrated Harvest with stabilizer to obtain the Drug Substance, which is further filled in a container closure system before storage

Manufacturing Process Development (DS)

The development was based on the experience gained from previous development of vaccines. The manufacturing process is based on CYD dengue virus culture and replication on Vero cells followed by several stages intended to achieve expected virus quality (filtration and purification stages) and yield (concentration stage) before filling the DS into bags. Scale up and industrial development were initiated very early on in the development program in parallel with the pre clinical and clinical phases. Three main product development phases can be distinguished:

- Phase I manufacturing process. Phase I process batches were used in phase I clinical studies and in non clinical studies. The objective was to deliver batches in order to test the proof of concept of the vaccine;
- Phase II manufacturing process. Phase II process batches were mainly used in phase II clinical studies and in non clinical studies. The objective was to define a final manufacturing process which can be then easily scalable and produced using human and animal-free origin materials;
- Phase III manufacturing process. Phase III process batches are currently used in phase II/III clinical studies and in non clinical studies. The objective was to increase the batch size while optimizing the process.

The comparability between development phases has been addressed by comparing materials which have been involved in clinical trials. The comparability exercise was based on the principles set forth in ICH guideline "Comparability of biotechnological/biological products subject to changes in their manufacturing process Q5E". As a conclusion, data generated demonstrate that the scale up and the improvement of the manufacturing process do no adversely impact the product quality and characteristics of the CYD dengue Drug Substance.

Manufacturing Process Development (DP)

CYD dengue vaccine is presented as a white homogeneous freeze dried product for reconstitution in a single dose or five dose glass vial and is a sterile preservative free formulation. Each dose contains 0.5 mL of CYD dengue vaccine after reconstitution with the diluent (0.4% NaCl for single dose and 0.9% NaCl for multidose (DENGVAXIA MD)).

CYD dengue vaccine is formulated with the 4 CYD dengue Drug Substances and excipients. The excipients are the components of the stabilizer. The excipients are needed for stabilization of the freeze dried pharmaceutical form of the vaccine. The development of the

vaccine composition mainly focused on determining optimal physicochemical parameters of the formulation, and to select the appropriate excipients composition to maintain the vaccine quality during the freeze drying process and throughout the freeze dried product shelf life.

The primary focus of the development program was to provide a safe and immunogenic product that maintains its quality throughout the shelf life and in a form that is easily administered, suitable for an industrial manufacturing process and for clinical and commercial handling. Scale up and industrial development were initiated very early on during the development program in parallel with the preclinical and clinical phases. Three main product/process development phases can be distinguished: starting with phase I and continuing through phase III:

- Phase I process development proposed a frozen liquid filled product stabilized by means of Human Serum Albumin (HSA) and lactose. The objective was to deliver batches in order to test the proof of concept of the vaccine.
- From phase II, process development targeted a more convenient pharmaceutical form for handling and storage and a manufacturing process which is then easily scalable. The choice was to develop a freeze dried Drug Product (DP) to be reconstituted prior to injection. The development had focused on the selection of excipients compatible with CYD dengue viruses, the use of human and animal free origin components and which are able to maintain virus properties during the manufacturing process and along the DP shelf life.
- For phase III process development, the main change was a batch scale increase for the FBP and DP as an adjustment to an industrial scale to fit the needs of the clinical development program and anticipate commercial production capacity. The sterile final filtration has evolved from tank to tank filtration to the implementation of on-line filtration for the commercial batches in order to carry out the final sterile filtration as close as possible to the filling point.

The comparability of CYD dengue DP throughout these development phases was evaluated by comparing phases I, phase II and phase III process batches. Representative clinical batches and representative batches of future commercial manufactured lots have been retained for the comparability exercise. The comparability exercise is based on the principles set forth in ICH Q5E: Guideline on comparability of biotechnological/biological products subject to changes in their manufacturing process. As a conclusion, data generated demonstrate that the scale up and the improvement of the manufacturing process did not adversely impact the product quality of the CYD dengue Drug Product and that the product attributes are highly similar across the process development phases. In parallel, the comparability is supported by the lot to lot consistency and bridging study CYD17. This study is first designed to demonstrate that three consecutively manufactured phase III process batches induce an equivalent immune response in terms of post dose 3 Geometric Mean Titers (GMTs) against the four parental serotypes, then to demonstrate that data from one phase II process batch and pooled data from the three consecutively manufactured phase III process batches show an equivalent immune response. The study results are presented in the clinical section

The virus potency is the main biological properties of the Drug Product and is insured by the measurement of CYD dengue virus concentration. Sterility is an important property to achieve for injected vaccines. Sterility is ensured by means of a validated aseptic process simulation and a validated sterilizing filtration process. The physicochemical properties (e.g. appearance, pH, osmolality) of the Drug Product are associated to the excipients and diluent used. Virus concentration, sterility and physicochemical properties of the vaccine are assessed by the release tests. Particles and filaments have been observed at both DS and DP stages on CYD dengue samples and are the result from the aggregation of endogenous proteins (i.e. proteins of Vero cell origin).

The compatibility between the CYD dengue viruses and the chosen excipients is demonstrated by the stability studies performed under normal and accelerated conditions in accordance with ICH guideline "Stability testing of biotechnological/biological products Q5C". Similarly, the compatibility between the Drug Product and its corresponding diluent for reconstitution has been also tested as part of the stability program.

Active Substance

Active Drug Substance: Each dose (0.5 ml) Contains

CYD dengue virus serotype 1	4.5-6.0 log10 CCID50/dose
CYD dengue virus serotype 2	4.5-6.0 log10 CCID50/dose
CYD dengue virus serotype 3	4.5-6.0 log10 CCID50/dose
CYD dengue virus serotype 4	4.5-6.0 log10 CCID50/dose

Manufacturers

The manufacture of the CYD dengue **Drug Substances** (**DS**) is performed at:

- Sanofi Pasteur: 1541 avenue Marcel Mérieux, 69280 Marcy l'Etoile, France

The QC testing of the DS is undertaken at the following sites:

- Sanofi Pasteur: 31-33 quai Armand Barbès, 69250 Neuville-sur-Saône, France
- Sanofi Pasteur: 1541 avenue Marcel Mérieux, 69280 Marcy l'Etoile, France

The manufacture of the CYD dengue **Drug Product (DP)** is performed at the following sites as shown in the table:

Manufacturing Facility	Operations
Sanofi Pasteur S.A., Parc Industriel d'incarville 27100 Val de Reuil, France	• Freeze-dried vaccine: manufacture, QC testing, batch release, Labeling and secondary packaging, Final batch release
Sanofi Pasteur S.A., Campus Merieux 1541 avenue Marcel Merieux 69280 Marcy l'Etoile, France	• Freeze-dried vaccine: QC testing
Sanofi Pasteur S.A., 31-33 Quai Armand Barb.s 69250 Neuville-Sur-Sa.ne, France	• Freeze-dried vaccine: QC testing

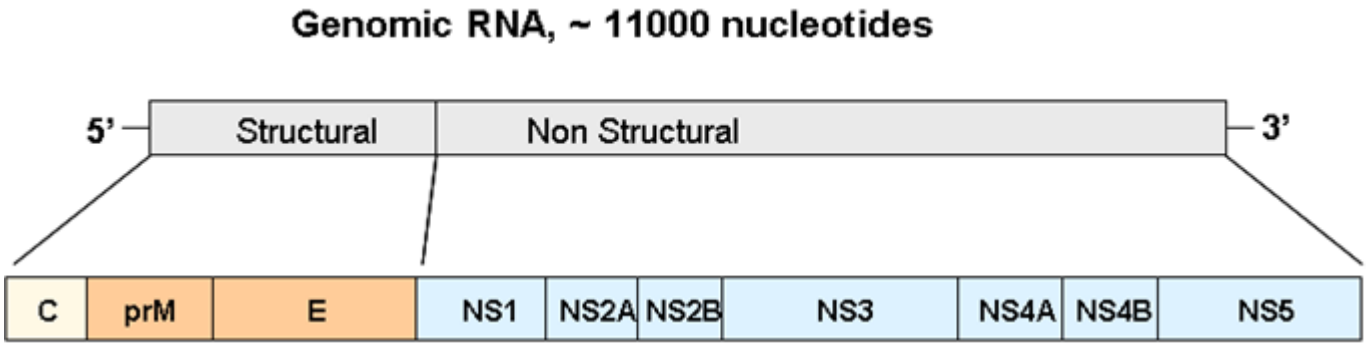
Structure of Seed virus

The 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.

The YF-17D virus and the wt dengue virus serotypes 1--4 are members of the flavivirus genus of the *Flaviviridae* family. Albeit being chimeric, the structure of CYD virions and their mode of replication in infected cells are the same as other flaviviruses.

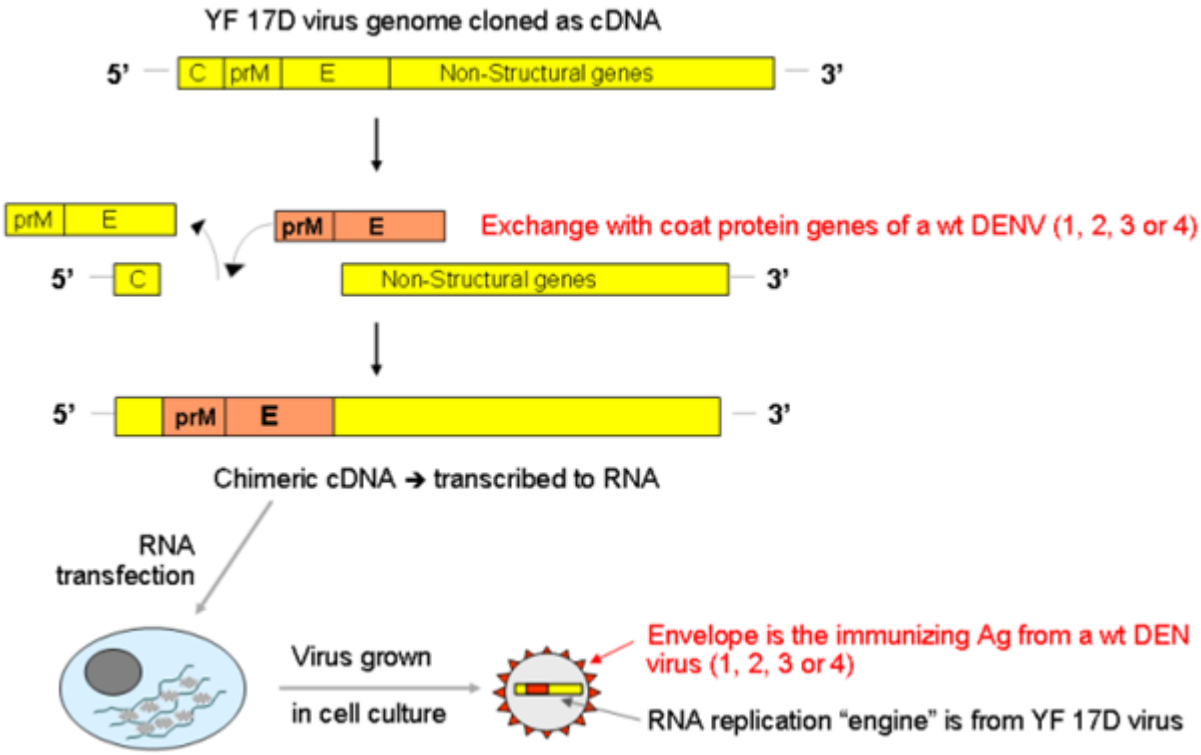
The flavivirus particles have a diameter of approximately 50 nm and contain a positive-sense, single-stranded RNA genome. The RNA genome encodes the structural and the non-structural proteins in a single open reading frame ~~(see Figure 1)~~. The 5' end of the viral genome contains three structural proteins: the capsid (C) protein, the pre-membrane (prM) and envelope (E) proteins. The 3' end of the viral genome contains seven non-structural (NS) proteins that consist of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

Figure ~~3~~ 2: Flavivirus Genome Structure and Polyprotein Processing



The E protein is the primary surface structural protein. It contains the antigenic determinants that define protective immunity (neutralizing epitopes) and is essential for membrane fusion and binding to cellular receptors

Figure ~~4~~ 3: Construction of Recombinant Complementary Deoxyribonucleic Acid (cDNA) of each CYD Dengue Virus



The TFDA recommended on The Quality Dossiers as the followings:

Summary of assesments

Major objections

DRUG SUBSTANCES:

1. The expert has reviewed and assesed the development of stabilizer, VP-SFM AGT and their components, Recombinant Trypsin, manufacture, quality control including with the test result in order to confirm the safety.

2. The expert has requested, reviewed and evaluated the validation method of the Virus concentration and Virus identification and also asked if appropiate internal controls prepared from Clinical lots are used and calibrated with internal controls material (s) used in testing of the batch manufactured. The manufacturer has responded that internal validity control are used in such tests.

3. The expert has reviewed the source of vero cell, MRC-5 which is used in extraneous agents using cell in control cell of supernatant, list of standard materials and list of references used in control test of DS, source, manufacture, specification, stability studies for storage and shelf-life. COA were also provided.

4. The expert has reviewed and assesed the container closure system: description of container and closure, type of primary packaging used including specification and testing method, appropriateness of material selection for DS, for example, compatibility of material with DS both physico- chemical aspect, leachable and extractable substances.

5. The expert has reviewed the Characterization of Drug substance. There were studies of Characterization of Drug substance by Genotypic (sequencing) and Phenotypic (neurovirulence) aspects. All the datas were already submitted.

DRUG PRODUCT:

1. The expert has reviewed and assesed the appearance of drug substance and drug product. Occasional endogeneous particles and filaments have been observed at both DS and DP stages on CYD dengue samples, and are the result from the aggregation of endogenous proteins (i.e. proteins but the levels of protein are very low in the final product). The manufacturer agreed that the presence of these particles is considered acceptable and it has been included in the DP release specification. From non-clinical and clinical studies, there were no reporting of toxicities and no impacts to immunogenicity. Moreover, the manufacturer has already proposed a pharmacovigilance (PV) plan which is acceptable.

2. The expert has reviewed and assessed the list of standard materials and reference standards used in control test of DP, certificate of analysis, manufacture method, control test, specification and stability monitoring, storage condition and shelf-life.

Other concerns

Drug substance:

1. Stability result of WSL: pending data at 132 months for serotype CYD-1,2, 120 and 132 months for serotype CYD-3 and 108, 120 and 132 months for serotype CYD-4. It was requested to the manufacturer to submit the complete data once the study is over

2. Transportation validation of CYD Dengue DS at <-70°C. The validation of temperature and duration of transportation is in specification.

3. Analytical Procedures of each testing method are available including the test which was exempted.

Drug product:

1. The manufacturer submitted all analytical procedures, validation of analytical procedures as expert requested such as sterility, virus concentration, virus identification, Thermal stability, appearance of freeze-dried product, appearance after dissolution, residual moisture, dissolution time, pH, osmolality, bacterial endotoxin and abnormal toxicity

2. Stability study of vaccine. Long term stability data is available under storage condition 2-8°C for 36 month and after reconstitution, the vaccine could be kept until **6 hrs** under storage condition 2-8°C. Stability study under accerelated conditions, i.e. Stability Study at 25+/- 2°C for

3 to 6 months, is also available. The manufacturer commits to perform the annual stability studies of industrial batch of CYD dengue vaccine at least 1 batch/ year for 3 consecutive years, to follow up the quality of the vaccine until the end of shelf life.

3. Diluent : 0.4% (for Dengvaxia) and 0.9%(for Dengvaxia MD) sodium chloride solution. The manufacturer has submitted the complete data of analytical procedures, validation of analytical procedures of all test methods according to expert request such as Sodium chloride content, Sodium identification, container closure integrity test, particulate contamination subvisible particles, extractable volume including the stability of diluent

TFDA PTL AND EXTERNAL EXPERT'S OVERALL CONCLUSIONS ON QUALITY ASPECTS

The manufacturer has conducted the development and manufacture vaccine in accordance with different related guidelines such as WHO Guidelines (e.g. Guidelines for the production and quality control of candidate tetravalent dengue virus vaccines (live), WHO TRS no. 910, 2002; Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated), WHO TRS no. 979, 2013, Annex 2 -Replacement of Annex 1 of WHO Technical Report Series, No. 932-). The GMP status of the vaccine manufacture is confirmed by GMP certificates issued by The National Agency for the Safety of Medicines and Health Products (L'Agence nationale de sécurité du médicament et des produits de santé or **ANSM**).

The company committed to continue the stability studies according to the protocol until completion of the study.

In parallel, the company committed to continue safety monitoring of vaccine according to the Pharmacovigilance plan attached and commented by the clinical expert.

2.3 Non Clinical aspects

Non clinical studies comprise of

- Pharmacological study program to evaluate primary pharmacodynamics and safety

-Toxicological studies to evaluate systemic and local toxicity, distribution and shedding, neurovirulence, developmental and reproductive toxicity and could be summarized as follows:

PHARMACOLOGY

1 Pharmacodynamics studies (immunogenicity of the vaccine)

The objective and result of CYD dengue vaccine studies are summarized as follows:

1.1 Assessment of immunogenicity and viremia: 7 studies addressed this point

1.2 Assessment of immunogenicity and protection to wild- type viremia: Two studies addressed this point.

According to WHO guidelines, it is important to monitor vaccine immunogenicity and efficacy in immunocompetent hosts. Since CYD dengue viruses replicate poorly in immunocompetent mice after subcutaneous or intramuscular immunization, this species has therefore not been used for pharmacological studies. The preclinical evaluation of the CYD dengue viruses and vaccine has been conducted in nonhuman primates (NHPs), including Rhesus (Macaca mulatta) and cynomolgus monkeys (Macaca fascicularis), given their susceptibility to infection by different flaviviruses, resulting in viremia and then immunogenicity. This provided useful information for vaccine development.

1.3 Assessment of the breadth of protection stimulated by CYD dengue: Four studies addressed this point

1.4 Evaluation of mitigation of interferences between 4 serotypes: Five studies addressed this point

1.5 Assessment of potential sensitization. Pre-existing flavivirus pre-immunity may theoretically sensitize to severe infection. Two studies addressed this potential risk.

The results of studies conducted in monkeys indicated that tetravalent CYD dengue vaccine is immunogenic and induces neutralizing antibody responses to all 4 dengue virus serotypes. Vaccine induced total or partial protection against wild type viremia upon mild or highly virulent challenge respectively.

After primary immunization, CD-4 consistently induced neutralizing antibody responses, which increased after following doses. Antibody responses were associated to viremia after CYD-4 immunization, but no such link was seen for CYD-3, and CYD-1 induced high antibody levels in absence of viremia. The studies in monkeys also indicated that multi-dose or alternative prime-boost regimens could increase the level and breadth of immune responses. One has to notice

that the serotype dominance in monkey appears to be different from the one seen in humans, as vaccine serotypes 2 and 3 induce low responses in monkeys while this response is higher in humans. It also appears that responses induced by a first dose induce a longer in refractory period in humans than in monkeys.

Pre-existing immunity to a flavivirus (such as YFV 17D) or to any dengue serotype was shown to result in a broader and stronger response to tetravalent CYD vaccination without increase of viremia. Therefore, the response to tetravalent CYD vaccination can achieve stronger and broader responses in endemic areas or in areas where YF 17D vaccination is routinely implemented.

TOXICOLOGY

1. General Toxicity

To evaluate the general and local toxicity of CYD dengue vaccine, a repeat-dose toxicity study was conducted in cynomolgus monkeys given 3 doses of 0.5 ml human dose by SC route. The animals were observed up to 10-21 days after the third dose administration. There was no death, no adverse clinical signs, no injection site reactions, no ophthalmic findings, no change of serum chemistry, hematology, coagulation, urinalysis parameters, no macroscopic and microscopic findings, no change in organ weight, body weight and food consumption. All vaccinated monkeys showed an antibody response (by hemagglutination inhibition assay) to the 4 dengue serotypes following the 3 doses of CYD dengue vaccine.

2. Special toxicology for vaccines (when applicable)

The biodistribution study in cynomolgus monkeys evaluated the distribution, the persistence, elimination and shedding of dengue vaccine over a 3-, 9- and 21-day observation period after a single SC administration. Safety endpoints were also investigated to correlate any replication in tissues to potential toxicology changes.

2.1. Systemic and Local Toxicology

There were no premature deaths, no adverse clinical signs and no treatment-related changes in body temperature, body weight or food consumption, and no changes in serum chemistry, hematology, coagulation and urinalysis parameters. There were transient minimal to slight inflammatory reactions at the injection site. The vaccinated monkeys showed antibody response to at least one CYD dengue virus serotype at 21 days following SC vaccination. There was transient and low level of serum viral RNA in some monkeys. There was no shedding in body fluids. Viral RNAs, were found at the injection site, lymphoid tissues and liver at day 3 and day 9 following CYD dengue vaccination but not in the central nervous tissues. On day 21, there was very low level of RNA at the injection site and lymph nodes only which demonstrated the viral clearance.

2.2 Neurovirulence

The neurovirulence study was conducted in cynomolgus monkeys administered with the vaccine by the intracerebral route (IC) and comparing CYD dengue vaccine and YF 17D vaccine which is used as reference vaccine. The result showed that CYD dengue vaccine was less neurovirulent than the YF 17D vaccine. There were no vaccine-related adverse clinical signs or change in food consumption and serum chemistry, hematology and urinalysis parameters, histopathological changes in organs/tissues besides central nervous system. All vaccinated monkeys showed an antibody response to the 4 dengue serotypes after the IC injection of CYD dengue virus.

2.3 Special immunological investigations

- Toxicology studies in special population (No study)
- Genotoxicity and carcinogenicity studies, when applicable (No study)
- Reproductive toxicity studies for vaccines to be administered to pregnant women or individuals of fertile age. There were developmental and Reproductive Toxicity studies including dose-ranging study and pivotal toxicity studies in rabbit and mouse which are summarized here below:

1. Developmental and Reproductive Toxicity Study **in Rabbit**

Rabbits received two intravenous (IV) injections before mating and three IV injections during gestation (on days of gestation (DG) 6, 12 and 27 (DGs 6, 12 and 27, respectively). Blood samples were taken from females, fetuses and pups for immunogenicity analysis. Robust anti-CYD antibodies against all CYD dengue virus 4 serotypes were detected in the serum of all treated dams and transfer to fetuses and pups was shown. There were no treatment-related premature deaths, no adverse clinical signs and no treatment-related changes in body weight and food consumption. There were no treatment-related effects on mating, fertility, organ weights, ovarian or uterine parameters or natural delivery parameters. There were no treatment-related fetal external, soft tissue or skeletal abnormalities and there were no treatment-related effects on pup survival, growth and development.

2. Developmental and Reproductive Toxicity Study **in Mouse**

Mice received a single IV injection during gestation (DG 6, 9 or 12) or during lactation (DL 14). The concentration in 0.5 mL corresponded to one human dose (approximately 5 log10 CCID50), an intermediate dose (approximately 6.5 log10 CCID50), or the highest feasible dose (approximately 8 log10 CCID50). There were no premature deaths and no adverse clinical signs at highest dose level. There was reduction in body weight gains and food consumption in females given an intermediate dose. Fetal changes were limited to reduction in fetal body weights but no fetal abnormalities.

The slightly reduced skeletal ossification averages that occurred in litters of females given highest doses were considered not to be adverse. Viral exposure was detected with no evidence of transfer to fetuses. Some antibody response in dams and transfer to fetuses was also shown

Under the conditions of these 2 studies given IV, the maternal and developmental No Observed Adverse Effect Level (NOAEL) for CYD dengue vaccine was established at 5 log10 CCID50. Following the non-clinical studies submitted, there are sufficient data showing safety and efficacy of CYD vaccine for acceptance.

2.4 Clinical aspects

Efficacy summary

Efficacy data were obtained from CYD 14 which included 10,275 subjects aged 2-14 years-old from 5 countries in Asia Pacific, and CYD 15 which included 20,869 subjects aged 9-16 years old from 5 countries in Latin America and Caribbean over a 2-year period. From the pooled analyses of CYD14 and CYD15 studies, vaccine efficacy (VE) was demonstrated in all 4 serotypes of dengue viruses in subjects group aged 9-16 years old. The overall vaccine efficacy was 65.6% (95% CI: 60.7;69.9) against the symptomatic form of dengue regardless of severity and due to any serotype, and VE against serotype 1,2,3 and 4 was 58.4%,47.1%,73.6% and 83.2%, respectively. The vaccine can reduce the severe dengue disease for 92.9 (as per 1997 WHO classification)-93.2% (as per IDMC criteria) and hospitalization for 80.8%

The immunogenicity data from phase II studies showed that subjects aged over 17 years old responded well to the vaccine i.e. demonstrated high immunogenicity after 3-dose vaccination. The immunogenicity studies conducted in endemic areas in adults aged 18-45 years old, CYD 22 (Vietnam, N=20, aged 18-45 yrs old) and CYD 47(India, N=126, aged 18-45 yrs old), showed that the levels of GMT to all 4 serotypes post dose 3 were higher than those observed in children from CYD 14 and CYD 15 where VE was demonstrated. It could be concluded that the vaccine efficacy in adults aged 17-45 years old can be as of that found in older children.

Safety summary

The vaccine was investigated through phase I- III clinical studies and had been administered in approx. 40,000 subjects in 15 countries. There were approximately 29,000 children, adolescent and adult who received the vaccine and demonstrated that vaccine has an acceptable safety profile.

The safety data from the active surveillance of the clinical studies CYD 14 and CYD 15 (i.e. for 25 months from first injection) showed that the adverse reaction in the vaccine groups were not different from those of in the placebo groups

According to the safety data analysis from 13 clinical studies which included subjects aged 9-60 years 20,667 that received dengue vaccine and 9,833 subjects that received placebo, all subjects who received at least 1 dose showed that the local and systemic adverse reactions were not different between vaccine and placebo groups, both in subjects aged 9-17

years old and subjects aged 18-60 years old. In conclusion, dengue vaccine has acceptable safety profile, i.e. comparable to placebo, in subjects aged 9-60 years old.

Immunogenicity summary

In the subjects aged 2-16 years old, the immune response level for all serotypes was good from the first vaccination and increased with the subsequent doses. In phase II study, it was found that subjects over 17 years of age had higher immunogenic response to vaccination.

The immunogenicity in adult aged 18-45 years old in endemic area showed that GMT level to all 4 serotypes were higher than in the children subjects in CYD 14 and CYD 15 where VE was demonstrated. In subjects aged 46-60 years old from non-endemic areas, the vaccine is safe and neutralizing antibody levels increased after the 3 doses schedule similarly to subjects aged 18-45-year-old.

Consideration for immunological response to natural dengue infection and vaccine safety.

The natural dengue viral infection will activate the immune system to produce **homotypic antibody** which will be lifelong immunity. However, in the same infection the body may also produce high level of the **heterotypic antibodies** which in time, these antibodies will decline and may facilitate the viral transmission into the cell and cause severe dengue. This phenomenon is called antibody-dependent enhancement (ADE) and is assumed to be one of the cause of severe dengue in population that has heterotypic antibody.

Such hypothesis leads to the concern that the dengue vaccine may theoretically lead the vaccinee to have severe dengue. However, this theory has no clinical evidences in humans and has evidence only from in vitro study. To date, there was no important difference in clinical signs and symptoms in hospitalized VCD cases between vaccine and placebo recipients and between the active and the long-term-term safety follow-up phase: i.e. no increase in severity, duration and frequency of clinical symptoms and length of hospitalization. Among hospitalized cases, no increase in viremia level and no cytokine pattern associated with ADE were observed between the active and hospital phase and between those who received the vaccine and the placebo.

Consideration:

1. The efficacy of vaccine post dose 3: the manufacturer will further continue the study to obtain more data on long-term efficacy

2. Follow up after year 2: There is a trend for more children aged 2-5 years hospitalized than placebo (relative risk (RR) of hospitalized severe and non-severe VCD case of 7.45 (95%CI:1.15;313.80) during Y3 and overall RR of 1.138 (95%CI: 0.59;2.31) during the entire study period). The manufacturer has confirmed to continue the studies to acquire more data until 5 years after complete vaccination which has already been explained in Risk Management Plan (RMP).

3. Co-administration with other vaccines: The manufacturer has planned to initiate the studies in 2016 with Tdap, HPV, and Influenza vaccines.

The expert has reviewed the manufacturer's responses and agreed that the data is clear and the manufacturer will continue to study as per the proposed plan which is acceptable.

Conclusion:

According to the result of assessments from chemical and pharmaceutical, pharmacology, toxicology and clinical documentations, it is found that Dengvaxia / Dengvaxia MD has acceptable quality, efficacy and safety profiles for registration approval by TFDA 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 with the Safety Monitoring program (SMP) follow up for 2 years and continue with Risk Management Plan as submitted to TFDA.

Reference:

[1] source from WHO fact sheet (http://www.who.int/mediacentre/factsheets/fs117/en/)

[2] source from WHO (**http://www.who.int/entity/wer/2016/wer9130.pdf**)