Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Development of the Sanofi Pasteur tetravalent dengue vaccine: One more step forward

Bruno Guy[a,*], Olivier Briand[b], Jean Lang[a], Melanie Saville[a], Nicholas Jackson[a]

[a] Research and Development, Sanofi Pasteur, 69007 Lyon, France
[b] Industrial Operations, Sanofi Pasteur, 69007 Lyon, France

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Dengue
Tetravalent dengue vaccine
Vaccine
flavivirus

ABSTRACT

Sanofi Pasteur has developed a recombinant, live-attenuated, tetravalent dengue vaccine (CYD-TDV) that is in late-stage development. The present review summarizes the different steps in the development of this dengue vaccine, with a particular focus on the clinical data from three efficacy trials, which includes one proof-of-concept phase IIb (NCT00842530) and two pivotal phase III efficacy trials (NCT01373281 and NCT01374516). Earlier studies showed that the CYD-TDV candidate had a satisfactory safety profile and was immunogenic across the four vaccine serotypes in both *in vitro* and *in vivo* preclinical tests, as well as in initial phase I to phase II clinical trials in both flavivirus-naïve and seropositive individuals. Data from the 25 months (after the first injection) active phase of the two pivotal phase III efficacy studies shows that CYD-TDV (administered at 0, 6, and 12 months) is efficacious against virologically-confirmed disease (primary endpoint) and has a good safety profile. Secondary analyses also showed efficacy against all four dengue serotypes and protection against severe disease and hospitalization. The end of the active phases in these studies completes more than a decade of development of CYD-TDV, but considerable activities and efforts remain to address outstanding scientific, clinical, and immunological questions, while preparing for the introduction and use of CYD-TDV. Additional safety observations were recently reported from the first complete year of hospital phase longer term surveillance for two phase 3 studies and the first and second completed years for one phase 2b study, demonstrating the optimal age for intervention from 9 years. Dengue is a complex disease, and both short-term and long-term safety and efficacy will continue to be addressed by ongoing long-term follow-up and future post-licensure studies.

1. Introduction

The month of November 2014 saw the successful achievement of a critical phase of an ambitious and challenging program that had been launched 20 years before, supported by an overall investment of more than one billion Euros, and which mobilized more than 1000 people in a large number of countries across different continents: for the first time in human history, a robot successfully landed on a comet. Despite difficulties and uncertainties, Philae and Rosetta so far have achieved most of their initial goals and have already brought a significant contribution to space exploration.

The development of the Sanofi Pasteur's recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine (TDV), often referred to as the CYD dengue vaccine or CYD-TDV, presents many similarities with the long-standing effort represented by the European Rosetta project, having started at the same time in the mid-90s, required a similar investment, and mobilized similar numbers of collaborators from multigeopgraphic and multidisciplinary teams, within and outside the company, and for which success could only be established at the end of development. While it still represents a large effort necessitating the acquisition of data in pivotal efficacy trials, the present review aims to summarize the different steps of this vaccine's development, focusing on the clinical data acquired in the last four years from three efficacy trials—one proof-of-concept phase IIb and two pivotal phase III efficacy trials. It will thus update the previous reviews published in *Vaccine* in 2010 and 2011 [1,2].

The initial steps of the preclinical and clinical development of CYD-TDV will be briefly covered, followed by a more detailed description and comparison of the three efficacy trials, where results of the active phase were obtained in 2012 and in 2014 [3–6].

Abbreviations: CYD-TDV or TDV, recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine; DENV-1–4, dengue virus serotypes 1–4; $PRNT_{50}$, 50% reduction dengue viral plaques as determined with the plaque-reduction neutralization test; WHO, World Health Organization.

* Corresponding author at: Research and Development, Sanofi Pasteur, 2 av. Pont Pasteur, 69007 Lyon, France. Tel.: +33 04 37 66 98 17.
E-mail address: bruno.guy@sanofipasteur.com (B. Guy).

http://dx.doi.org/10.1016/j.vaccine.2015.09.108

Beyond the observed results, these ongoing trials (now in the hospital surveillance period) have generated several questions and lines of investigations, which will be addressed. Finally, while a critical phase has now been completed, it is important to consider what remains to ensure implementation of the vaccine that will bring the highest benefit to human health, which will also be described.

2. Early development

Previous reviews have described in detail the results of initial investigations that aimed to characterize the CYD-TDV at both preclinical and early clinical development stages, in order to evaluate potential risks in line with a Development Risk Management Plan at the onset of CYD-TDV availability [1,2]. These investigations addressed the following points and questions, with conclusions presented in Fig. 1:

- Genetic and phenotypic stability [7–9].
- Post-translational modifications such as glycosylation [10].
- Preclinical evaluation of immunogenicity: *in vitro* [11–13] and *in vivo* [14–16].
- Non-clinical safety [17].
- Theoretical risks: transmission by arthropod vectors [18,19], reversion to virulence [20], recombination with a wild type (flavi)virus [21–23], viscerotropism [11,24], sensitization/antibody-dependent enhancement (ADE) [25].
- Initial clinical evaluation: reactogenicity/safety and immunogenicity, addressing both neutralizing antibody [26–33] and T-cell responses [34–36].

These investigations demonstrated that the CYD-TDV candidate had satisfactory safety and immunogenicity in both *in vitro* and *in vivo* preclinical tests, as well as in initial phase I and phase II clinical trials in both flavivirus naïve and seropositive individuals. Furthermore, both humoral and cellular responses were induced in humans against all four dengue virus serotypes (DENV-1–4) of the vaccine.

These potential risks hypothesized as being associated with these chimeric vaccine viruses have been explored in depth. They have been identified based on current knowledge in the dengue field, and also in agreement with the European Medicines Agency (EMA) guidelines on recombinant vaccines [37]. A recently proposed standardized template for a risk-benefit assessment of vaccines based on a yellow fever backbone is a key consideration in the comparison of such vaccine technologies [38]. For each of the potential risks, a risk-minimization action plan was defined and data generated, which were subsequently reviewed with the World Health Organization (WHO), Pan American Health Organization, Centers for Disease Control and Prevention, key opinion leaders, and regulatory agencies.

3. Industrial development

Scale up and industrialization were initiated very early on in the development program—in parallel with the preclinical phase and clinical phase I—to ensure that the demand for the vaccine to be used in clinical phase III could be met, as well as the future demand for the licensed vaccine. The production process has been summarized previously [2], and was set up to ensure a reliable and consistent supply of virus and cells at the industrial level.

As early as 2006, the production process was transferred to Industrial Operations and a production facility was dedicated at the Marcy L'Etoile site that was equipped with industrial-scale biogenerators to produce Vero cells and virus. Briefly, the four vaccine viruses are produced from four virus seed lots using an identical manufacturing process for each serotype. Banking systems for serum-free Vero cells were established to produce master and working viral seeds and cells, allowing reliable and consistent supply of virus and cells, respectively. The vaccines and cells are characterized and tested for safety in accordance with WHO, European and US guidelines [39]. All the tests undertaken are part of a control strategy designed to ensure product quality and consistency. These included quality control specification, product characterization, adherence to good manufacturing practices, validated manufacturing process, raw-materials testing, in-process testing, and stability testing. The quality control specification, which is typical for a live, attenuated, viral vaccine, based on current regulations and guidelines, mainly determined the purity, safety, and potency of the vaccine [39]. Due to the use of Sanofi Pasteur's serum-free Vero cell banks for both cell and viral culture, the CYD-TDV manufacturing process includes no raw materials of animal origin; neither does the vaccine contain any preservatives, adjuvants, or antibiotics. A proprietary stabilizer is present in the finished product, which has been shown to have excellent stability: accelerated stability studies have shown that the vaccine from the phase III lots of CYD-TDV (unidose presentation) was stable up to 1 month at $25 \pm 2\,^{\circ}C$, and that the viral titer decreased by less than $0.5\ \log_{10}\ CCID_{50}$ (the 50% cell culture infective dose) after 7 days at $+37 \pm 2$ C. Reconstituted vaccine was found to be stable for up to 6 h at $+5 \pm 3\,^{\circ}C$.

In 2008, 4 years before the first results of the phase IIb clinical trials, it was decided to establish a new vaccine production site at Neuville sur Saone, France, in anticipation of future vaccine needs and to be able to minimize the vaccine-to-vaccination gap after regulatory approval. This represented a €300 million investment, consistent with the continuous efforts made by the Sanofi Pasteur teams over the past 20 years toward the development of a safe and efficacious dengue vaccine. Three new dedicated facilities (utilities, quality control, and production) have been built on this site using quality by design principles, and fitted with state of the art technology equipment. The Neuville sur Saone production facilities scales up the Marcy l'Etoile facility and can produce up to 100 million doses per year of vaccine virus (drug substance, packaged in monodose or multidose vials). The new site has gone through the qualification and validation steps, and consistent lots have already been produced at this site that will ensure the availability of the vaccine on an industrial scale over the coming years.

Another important Sanofi Pasteur site, in Val de Reuil, France, is also involved in the production of CYD-TDV. Here, the vaccine is formulated, filled, lyophilized, and packaged before release. The final product can then be shipped from Val de Reuil to all over the world (through distribution facilities).

4. Recent clinical development: phase IIb (CYD23) and phase III (CYD14 and CYD15) efficacy trials

The clinical development of CYD-TDV has complied with the International Conference on Harmonization guidance for industry, as well as the US Food and Drug Administration and European Medicines Agency guidelines for new vaccines, and the WHO Technical Report Series 932 guidelines for the production and quality control of live candidate tetravalent dengue virus vaccines [39]. This required consideration of specific development challenges (Fig. 1), in addition to the lack of a predictive immunocompetent animal disease model. These challenges include but are not limited to: (i) the need to induce an adequate immune response to all four serotypes; (ii) the current absence of a correlate and threshold of protection, and thus the need to demonstrate clinical efficacy; (iii) the need to demonstrate long-term safety; (iv) the potential risks after vaccination of sensitization to severe dengue infection and of

Please cite this article in press as: Guy B, et al. Development of the Sanofi Pasteur tetravalent dengue vaccine: One more step forward. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.09.108