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Dengue vaccine development: Global and Indian scenarios

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ABSTRACT

India is home to nearly a third of the global population at risk of dengue, a viral disease caused by four antigenically and genetically distinct dengue viruses. Clinical illness following dengue virus infection can either be mild and self-limiting dengue fever or severe dengue hemorrhagic fever/dengue shock syndrome, with potentially fatal consequences. A live attenuated vaccine known as Dengvaxia, developed by Sanofi, was licensed in 2015. Following this, long-term follow-up of the Sanofi phase III efficacy trial participants has revealed potential safety concerns. This vaccine, which appears to predispose dengue-naïve recipients to an increased risk of hospitalization in the future, is recommended by the World Health Organization only for adults with a history of prior dengue virus infection. A safe and efficacious dengue vaccine continues to be sought globally. India has joined these efforts in recent years, and is poised to initiate the clinical development of two candidates in the near future, one licensed from abroad and the other developed indigenously. This article provides a glimpse of India's efforts to develop dengue vaccines in the context of the global dengue vaccine development and evaluation landscape and highlights key issues and questions confronting the dengue vaccine community.

Introduction

The World Health Organization (WHO) considers dengue to be one of the fastest spreading arboviral diseases (WHO, 2013). Dengue is caused by any of four antigenically and genetically distinct mosquito-borne dengue virus serotypes (DENV-1, 2, 3, and 4) of the genus *Flavivirus*, family *Flaviviridae*. Each DENV serotype is further classified into multiple genotypes, manifesting intra-serotype nucleotide sequence divergence of up to 6%. The majority of DENV infections are silent, but approximately 25% of these result in clinically apparent disease, which may manifest as dengue fever, a self-limiting febrile illness, or more severe dengue hemorrhagic fever and dengue shock syndrome.

What is the burden of dengue disease? Based on data for the year 2010, the International Research Consortium on Dengue Risk Assessment, Management and Surveillance (IDAMS) estimated that there are about 390 million infections around the world annually, of which about 96 million are symptomatic cases (Bhatt et al., 2013). India is one of 128 countries worldwide that is affected by dengue. India's first dengue epidemic was reported in the early 1960s in the eastern region (Gupta et al., 2012). Although dengue became a notifiable disease in India in 1996, it is widely agreed that dengue cases are under-reported. Adjusting the national average number of officially reported dengue cases of 20 474 in India for under-reporting based on an empirical case study in a district of South India, the International Clinical Epidemiology Network computed the national annual average number of clinically diagnosed dengue cases to be nearly 5.8 million during the period 2006–2012 (Shepard et al., 2014). However, according to the IDAMS report, clinically apparent cases of DENV infection were estimated to be approximately 33 million in 2010 (Bhatt et al., 2013).

A unique feature of dengue is that initial infection (primary infection) by any one DENV serotype can offer protection (homotypic) against subsequent infection by that serotype alone. While such protection is generally presumed to be life-long, protection against heterotypic DENVs is transient. When such cross-protection wanes, a subsequent infection (secondary infection) by a different DENV serotype can actually result in severe dengue disease. Epidemiological evidence suggests that secondary infections correlate with an increased risk of severe dengue disease (Guzman et al., 1990, 2002). This, together with laboratory evidence, has led to the widely accepted hypothesis that heterotypic antibodies bind to DENVs and increase their uptake into cells of the monocytic lineage via their Fcγ receptors (FcγR)

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and drive up virus load, leading to severe dengue. In this context, it has been discovered that antibodies to the pre-membrane (prM) protein and the fusion loop (FL) epitope of the envelope (E) protein, primarily play a role in enhancement of DENV infection (Dejnirattisai et al., 2010). The occurrence of antibody-dependent enhancement (ADE) of dengue disease strongly supports the rationale underlying current dengue vaccine development efforts, which is to provide balanced and durable immunity to all four DENV serotypes (Murphy and Whitehead, 2011).

A live attenuated vaccine (LAV), chimeric yellow fever/dengue tetravalent dengue vaccine (CYD-TDV), developed by Sanofi (Hadinegoro et al., 2015), was first licensed in 2015 and is now licensed in several dengue-endemic countries. CYD-TDV, which is marketed under the name Dengvaxia, has not been licensed in India as the regulators required additional safety data. Two additional LAVs, the US National Institutes of Health (NIH) tetravalent dengue vaccine (TetraVax-DV) and Takeda's tetravalent dengue vaccine (TDV), are in phase III efficacy trials at the current time. India has initiated its own efforts to develop two dengue vaccine candidates (Figure 1). One is a LAV, TetraVax-DV, licensed from the US NIH to Indian pharmaceutical companies (Access to Vaccines, 2017), and the other is an indigenously developed protein-based tetravalent dengue subunit vaccine, DSV4 (Ramasamy et al., 2018). This article will review Dengvaxia, which has completed phase III trials, and provide a snapshot of the current global dengue vaccine development landscape, a description of India's vaccine candidates and their current development status, and briefly highlight some key issues and questions.

Dengvaxia

Dengvaxia is a mixture of four monovalent chimeric vaccine viruses, CYD-1, CYD-2, CYD-3, and CYD-4, produced using Vero cells. All four CYDs are based on the yellow fever virus (YFV) 17D vaccine vector backbone, in which the genes encoding YFV structural proteins prM and E have been replaced by the corresponding genes of DENV-1, 2, 3, or 4. CYD-TDV was first licensed in late 2015 after an initial proof-of-concept single-center phase IIb trial in Thailand (CYD23 study), followed by two multicenter phase III efficacy trials in several Asian (CYD14 study) and Latin American (CYD15 study) countries. Collectively, these trials involved approximately 35 000 children aged 2–16 years. CYD-TDV, administered to human subjects in three doses (0, 6, 12 months), was shown to be well-tolerated and to elicit neutralizing antibodies (nAbs) against each of the four DENVs (Hadinegoro et al., 2015).

Interim data from long-term safety studies on CYD-TDV at the end of year 3 (post-dose 1), published in early 2015, revealed an increase in the number of hospitalized and severe dengue cases in younger children. An age-specific analysis of year 3 data from the CYD14 trial found that the relative risk (RR) of hospitalization in the 2–5 years age group was 7.45 (95% confidence interval (CI) 1.15–313.8). A safety follow-up study (designated CYD57) of the CYD23 trial subjects also reflected an elevated RR of hospitalization in 4–5-year-olds, the youngest age group in that study. A post-hoc analysis of year 3 data from all three trials (CYD14, CYD15, and CYD57) for children ≥9 years showed a RR of hospitalized dengue of 0.50 (95% CI 0.29–0.86). In children under 9 years of age (included in trials CYD14 and CYD57), the RR was 1.58 (95% CI 0.83–3.02). Based on these data, together with additional data relating to serostatus at baseline from Sanofi, the WHO Strategic Advisory Group of Experts on Immunization conditionally recommended the use of CYD-TDV in countries/regions with high levels of dengue transmission (seroprevalence ≥70%) for vaccinating populations in the 9–45 years age range.

Independent re-evaluation of the data, based on the premise that the hospitalized cases (seronegative prior to vaccination) in the vaccinated and placebo groups were immunologically different, strongly suggested that Dengvaxia sensitizes seronegative recipients to ADE upon subsequent DENV infection (Halstead, 2017). In late 2017, additional data from Sanofi over a duration of 5–6 years post-dose 1 corroborated this concern, providing evidence that seronegative recipients of Dengvaxia of all ages were more prone to severe dengue disease. This led the WHO Global Advisory Committee on Vaccine Safety to recommend that Dengvaxia be administered only to seropositive individuals (WHO, 2018). Recently Sanofi published year 4 follow-up data of its three trials mentioned above, CYD14, CYD15, and CYD57. The cumulative RR

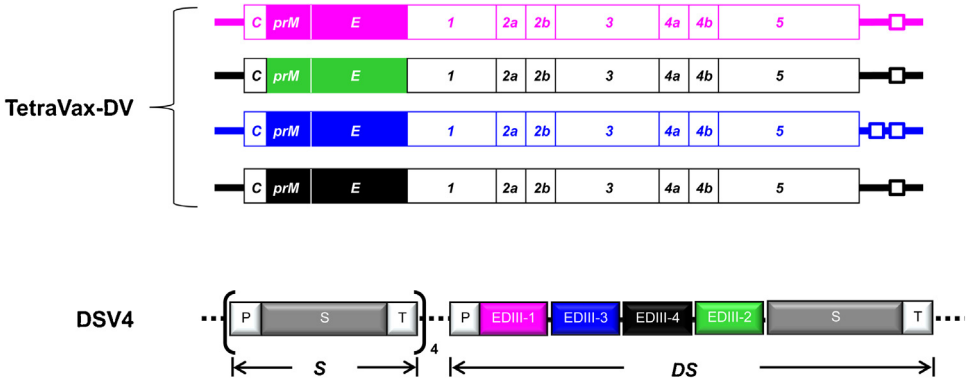

Figure 1. The two dengue vaccine candidates being developed in India. Shown at the top is a schematic representation of the set of the four RNA genomes of the attenuated DENV vaccine viruses that make up TetraVax-DV. The DENV serotypes of these vaccine strains, distinguished by different colors, derive from the parental serotype of their *prM* and *E* genes (shown by the filled boxes: magenta, DENV-1; green, DENV-2; blue, DENV-3; and black, DENV-4). The empty boxes denote the sequences encoding C (a structural protein of minor importance from the perspective of a vaccine) and the non-structural (NS) proteins (1, 2a, 2b, 3, 4a, 4b and 5). The short horizontal lines flanking the protein-encoding regions denote the 5′ and 3′ NTRs. These viruses were created by engineering a 30-nt deletion (indicated by the open square towards the right end of the genome) in their 3′ NTRs. In the case of DENV-3, a second 31-nt deletion, upstream of the initial 30-nt deletion, was required to achieve acceptable attenuation. In the case of serotype 2, acceptable attenuation required chimerization in addition to the 30-nt deletion, entailing the grafting of DENV-2 *prM* and *E* genes onto the attenuated DENV-4 genome (containing the 30-nt deletion in its 3′ NTR), in place of its own *prM* and *E* genes. Thus, the DENV-2 vaccine strain is a DENV-2/DENV-4 intertypic chimera. These vaccine viruses replicate in vivo and express viral antigens within the host. Shown on the bottom is a schematic representation of the DSV4 DNA integrated into the genome of the yeast *Pichia pastoris*. This DNA insert encodes four copies of the *S* gene, each with its own promoter (P) and terminator (T), and one copy of the chimeric fusion gene *DS* as an independent expression cassette. The *DS* gene is an in-frame fusion of sequences encoding EDIIIs of the four DENV serotypes (the same color-coding as used above to distinguish serotypes) plus the S protein. Upon co-expression in and co-purification from *P. pastoris*, the S and DS proteins co-assemble into mosaic VLPs. (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

for hospitalized virologically confirmed dengue in all of these trials over the 4-year follow-up period was 1.327 for Dengvaxia recipients who were seronegative at baseline, regardless of age at the time of vaccination (Arredondo-Garcia et al., 2018). It appears that Dengvaxia may have simulated a monotypic primary infection in seronegative vaccinees (Guy and Jackson, 2016). Consistent with this are recent data showing that Dengvaxia elicited antibodies predominantly specific to DENV-4 alone (Henein et al., 2017). This kind of skew in the antibody response to a LAV is likely the outcome of interference among the four monovalent vaccine viruses of the tetravalent formulation.

Lessons from Dengvaxia trials

Several insights have been provided by the experience with Dengvaxia. First, vaccine-induced nAbs per se may not serve as reliable surrogate markers of vaccine efficacy. Dengvaxia afforded the least protection against DENV-2 despite it eliciting high DENV-2 nAb titers. There is an urgent need to delineate the precise correlates of protection against dengue. Second, contrary to the prevalent belief in the dengue vaccine community that ADE is merely a theoretical concern, it is a real issue with LAVs (Halstead, 2017). Dengvaxia appears to have simulated a primary infection in subjects who were dengue-naïve at baseline, and predisposed them to severe dengue upon subsequent DENV infection. Third, it is necessary to differentiate between vaccine-induced type-specific and cross-reactive antibody responses and ensure that nAbs with type-specificity to all four DENV serotypes are induced by the vaccine. A recent study has determined that immune sera from Dengvaxia recipients neutralized DENV-4 alone by type-specific nAbs, with the remaining three DENV serotypes being neutralized by cross-reactive antibodies (Henein et al., 2017). This underlines the need to eliminate viral interference in LAVs to avoid the induction of immune responses predominantly to a single DENV serotype. Finally, it should be necessary to ensure that the new vaccines do not induce such cross-reactive, potentially DENV-enhancing antibodies.

Dengue vaccine candidates currently in clinical trials

The current status of various dengue vaccine candidates in clinical development are summarized in Table 1. These candidates include additional LAVs, purified inactivated vaccines (PIV), and recombinant protein and plasmid vaccines. All of these dengue

Table 1
Current status of tetravalent dengue vaccine candidates in clinical development[a].

Vaccine (type)	Sponsor	Trial identifier	Phase	Number (age range, years)	Site(s)	End date
TetraVax-DV[b] (LAV)	Butantan	NCT02406729[c]	III	16 944 (2–59)	Brazil	Dec 2022
Dengue tetravalent vaccine[d]	Panacea Biotec Ltd	CTRI/2017/02/007923	I/II	200 (2–60)	India	Not known
TDV[e] (LAV)	Takeda	NCT02747927[f]	III	20 100 (4–16)	Asia, Latin America	Dec 2021
TDENV (LAV)	WRAIR and GSK	NCT00239577	II	132 (18–45)	Maryland, USA	Jun 2007
		NCT00370682	II	120 (20–25)	Bangkok, Thailand	Feb 2008
		NCT00350337	II	88 (18–45)	Maryland, USA	Jul 2008
		NCT00468858[g]	II	636 (1–50)	Puerto Rico	Apr 2010
		NCT00384670	I/II	7 (6–10)	Bangkok, Thailand	May 2004
		NCT00322049	I/II	51 (1–1.25)	Bangkok, Thailand	Jun 2009
TDENV-PIV (Inactivated)	WRAIR and GSK	NCT02421367	I/II	140 (20–49)	Maryland, USA	Jun 2019
		NCT03141138[h]	I	40 (18–42)	Maryland, USA	Jan 2022
		NCT01666652	I	100 (18–39)	Maryland, USA	Sep 2018
		NCT01702857	I	100 (20–39)	Puerto Rico	Mar 2017
		NCT02239614[h]	I	80 (18–49)	Maryland, USA	Feb 2017
TVDV (DNA)	WRAIR and NMRC	NCT01502358	I	40 (18–50)	Maryland, USA	Dec 2013
V180 (r-protein)	NIAID and MSD	NCT02450838[i]	I	20 (18–50)	Maryland and Vermont, USA	Oct 2015
	MSD	NCT01477580	I	98 (18–49)	Unknown	Dec 2014

LAV, live attenuated vaccine.
[a] Compiled from data gleaned from the US-based global clinical trials registry at ClinicalTrials.gov., except the second row, which represents information from the Clinical Trials Registry–India website (http://ctri.nic.in); the most advanced trials (completed/ongoing), as on date for each of the vaccine candidates are shown.
[b] The NIH LAV is also known in Brazil as Butantan-DV.
[c] Multicenter trial at 16 sites in a single dengue-endemic country.
[d] This vaccine is the same as TetraVax-DV (NIH); this trial is yet to commence recruitment of volunteers.
[e] The Takeda vaccine is also known as TAK-003.
[f] Multicenter trial at 25 sites spread across eight dengue-endemic countries.
[g] Multicenter trial at 11 sites.
[h] TDENV-PIV has been/is being assessed in conjunction with TDENV-LAV (F17) in prime/boost immunization in these two trials.
[i] V180 was assessed in conjunction with NIH TetraVax-DV in a prime/boost immunization in this trial.

vaccine candidates seek to target all four DENV serotypes, and all rely on the DENV structural antigens, prM and E mentioned above, to elicit DENV nAbs.

The NIH LAV, TetraVax-DV, which is a mixture of four monovalent attenuated DENVs, is depicted in Figure 1 (Durbin et al., 2011). The attenuation is based on a targeted 30-nucleotide (nt) deletion (Δ30) in the 3′ non-translated region (NTR) of the DENV genomes of the four monovalent vaccine viruses. A phase III efficacy trial of TetraVax-DV is currently underway in several sites in Brazil, involving approximately 17 000 subjects including children, adolescents, and adults. In parallel, this vaccine is being tested in phase II trials, also in Brazil (NCT01696422). Additional phase II trials of TetraVax-DV are also ongoing in Thailand (NCT02332733), Taiwan (NCT03485144), and Bangladesh (NCT02678455). All of these phase II trials are scheduled to be completed in the next 1–2 years, ahead of completion of the phase III trial.

The Takeda vaccine, TDV, is once again a mixture of four monovalent chimeric LAVs, with all possessing the same DENV backbone, empirically attenuated by serial passaging of DENV-2 in primary dog kidney (PDK) cells (Osorio et al., 2016). TDV has completed several phase I and phase II studies and has been reported to be immunogenic and well-tolerated in children and adults. Two additional phase II studies are underway in multiple Asian and Latin American countries. These are expected to be completed in the latter halves of 2019 (NCT02302066) and 2020 (NCT02948829) and are intended to test single-dose versus two-dose vaccination regimens, respectively. A large multicenter phase III efficacy study in approximately 20 000 children, spread over several sites in the Philippines, Sri Lanka, Thailand, Brazil, Colombia, Dominican Republic, Nicaragua, and Panama, was initiated last year.

The US Walter Reed Army Institute of Research (WRAIR), in collaboration with GlaxoSmithKline (GSK), has been pursuing the development of a tetravalent LAV (TDENV-LAV), based on the empirical attenuation of each one of the four DENV strains by passaging them in PDK and fetal Rhesus lung cells, which are semi-permissive for DENV replication, and assessing them in phase I trials. The occurrence of viral interference necessitated the testing of multiple empirical tetravalent formulations of TDENV-LAV, resulting in the identification of formulation 17 (F17) as the lead candidate based on a phase II trial in US adults. Additional studies suggested that TDENV-F17 had an acceptable safety and immunogenicity profile. However, long-term follow-up revealed that humoral immunity was not durable. Results published more recently reveal that the type-specific nAb response elicited by this vaccine is restricted only to DENV-2 and DENV-4 (Gromowski et al., 2018).

WRAIR and GSK have also been developing another tetravalent vaccine candidate, TDENV-PIV, based on purified inactivated DENVs. Two phase I trials, testing different doses with three different adjuvants, have been completed and the results support continued clinical development of TDENV-PIV (Diaz et al., 2018; Schmidt et al., 2017). Another TDENV-PIV/TDENV-LAV prime/boost study has been concluded recently, but results are yet to be made available.

The WRAIR, together with the US Naval Medical Research Center (NMRC), has developed a plasmid DNA vaccine known as tetravalent dengue vaccine, TVDV. This vaccine contains a mixture of four plasmids, with each one encoding the *prM* and *E* genes of one DENV serotype. Data from a phase I trial of TVDV, which was completed in 2013, have just been reported recently. It was found that TVDV elicited predominantly anti-DENV T-cell interferon-gamma responses in a dose-dependent manner (Danko et al., 2018).

Merck Sharpe & Dohme (MSD) is developing a recombinant protein vaccine. This initiative, which was started by Hawaii Biotech, is based on insect cell-expressed C-terminally truncated versions of the DENV E proteins. Recently, Merck published the data from a phase I trial of the monovalent DENV-1 E protein (Manoff et al., 2015). A phase I trial of V180, a mixture of the four C-terminally truncated DENV E proteins, was concluded in 2014. Collaborating with the US National Institute of Allergy and Infectious Diseases (NIAID), MSD completed a phase I trial in 2015. In this trial, subjects who had previously been vaccinated with the NIH TetraVax-DV and had been seroconverted to at least three DENV serotypes were boosted with V180 with or without alum as adjuvant. No data on these two trials have been made available as yet.

LAV licensed to Indian companies from NIH

The Indian vaccine producers Panacea Biotec, Serum Institute, and Biological E have secured non-exclusive licenses for the clinical development and commercialization of the dengue vaccine TetraVax-DV developed by the US NIH (Access to Vaccines, 2017). To date, none of the Indian vaccine manufacturers has initiated phase I trials. However, phase I trial data from studies conducted in US populations are available and are reviewed below.

Currently the NIH is pursuing two formulations of TetraVax-DV, designated as TV003 and TV005 (Whitehead, 2016). Both are tetravalent formulations containing clinically evaluated monovalent Δ30 vaccine viruses, corresponding to the four DENV serotypes. After one dose of TV003 and TV005, 75% and 77%, respectively, of the vaccinated subjects manifested viremia. A majority of these (>80%) also developed a vaccine-associated rash, indicating vaccine 'take'. Following a second dose given 6 months later, there was neither viremia nor rash in any of the TV003 recipients, while there was one instance of viremia in the TV005 group. Mean antibody titers to each DENV serotype after the first and second doses of TV003 and TV005 were within two-fold of each other. This has been interpreted to reflect nearly complete sterilizing immunity induced by a single dose of TV003/TV005.

The NIH investigators have developed a DENV-2 human challenge model using the under-attenuated monovalent rDENV-2Δ30 virus, in a trial involving 10 healthy flavivirus-naïve volunteers (NCT01931176), which was excluded early on as a vaccine candidate. This virus is reported to have resulted in viremia in all ($n = 10$) and rash in 80% of challenged flavivirus-naïve volunteers, with a mean peak titer of 2.5 $\log_{10}$ PFU/ml. TV003 vaccinated subjects ($n = 21$) were completely protected against viremia and rash when challenged 6 months post-vaccination with 10^3 PFU of rDENV-2Δ30 virus, whereas all placebo recipients ($n = 20$) developed viremia with 80% of them manifesting rash (Kirkpatrick et al., 2016).

All human trials of TV003/TV005 above were conducted in flavivirus-naïve subjects. A recent study has compared the performance of TV003 in flavivirus-experienced subjects as well (Whitehead et al., 2017). These were healthy American adults (18–50 years) who had been either exposed to a prior flavivirus infection or had been the recipient of either a licensed flavivirus vaccine (against YFV or Japanese encephalitis virus) or an experimental DENV vaccine. This trial revealed that after one dose of TV003, 87% of the recipients mounted antibody responses against all four DENVs and 76% were viremic. However, a comparison of the data with those from an earlier trial of TV003 on flavivirus-naïve subjects showed that the frequency of tetravalent response was greater in subjects who were flavivirus-exposed prior to vaccination.

Data at hand from the TetraVax-DV phase I trials as well as upcoming data from the ongoing phase II and phase III trials of the NIH vaccine in the Asian countries and in Brazil will serve as a valuable reference for the clinical trial data anticipated to be

generated by the Indian companies in the near future. Panacea Biotec, which received the monovalent Δ30 virus strains during 2006–2007 from NIH, has completed preclinical studies, prepared vaccine material in accordance with current good manufacturing practice (cGMP) guidelines, and secured the Indian National Regulatory Authority's approval to conduct human trials. The company entered into an agreement with the Technology Development Board of India in November 2017 (TDB-Panacea Agreement, 2017) to complete late-stage development of Tetra-Vax-DV, and is planning to initiate an age de-escalation phase I/II trial soon. This trial plans to recruit 200 healthy volunteers in the age group 2–60 years spread across three sites in North and South India to evaluate the safety, reactogenicity, and immunogenicity of one dose of TetraVax-DV (Table 1). Panacea Biotec anticipates the vaccine to be launched in 2020. Serum Institute of India Limited, which secured an independent license from NIH in 2015 to develop TetraVax-DV in India, is currently conducting preclinical toxicity studies. Seed lots of the vaccine viruses have been prepared and characterized as per WHO TRS 979 requirements and cGMP manufacture is underway. The company anticipates initiating phase I testing early next year and phase III efficacy trials in 2020, with a 5-year follow-up, before seeking licensure. A third Indian company, Biological E, is also reported to have licensed the LAV from NIH, but no information is available on its development status.

Dengue subunit vaccine being developed indigenously

Unlike the four-component LAVs reviewed above, the tetravalent dengue subunit vaccine, DSV4, being co-developed by the International Centre for Genetic Engineering and Biotechnology (ICGEB) and Sun Pharma, is a single-component, non-replicating '4-in-1′ vaccine based on a virus-like particle (VLP) platform, produced using the methylotrophic yeast *Pichia pastoris* (Figure 1). The VLP platform is provided by the hepatitis B virus surface antigen (HBsAg). DSV4 contains HBsAg (referred to as the S protein) as well as a second protein, DS, co-assembled together into mosaic VLPs. DS is a chimeric protein, containing a unique tetravalent dengue antigen (D), fused in frame to HBsAg (Ramasamy et al., 2018). The D component of the DS protein is by itself a chimeric protein created by linking unique carboxy terminal domains derived from the E proteins of each of the four DENV serotypes, in frame with each other, in a head-to-tail tandem array. The unique domain is known as E domain III (EDIII). Each EDIII, which is approximately 100 amino acid residues long, is critical for DENV entry into susceptible cells during infection and elicits potent serotype-specific DENV nAbs. That DSV4 VLPs do display serotype-specific EDIII epitopes of all four serotypes has been verified using a panel of 20 well-characterized monoclonal antibodies. Preclinical proof-of-concept data supporting the potential of DSV4 as a dengue vaccine candidate, reviewed below, have been published recently (Ramasamy et al., 2018).

DSV4 has been found to be immunogenic in multiple strains of mice, eliciting high levels of nAbs capable of potently blocking the infection by each of the four prevalent DENV serotypes. It has also been found that the murine nAbs elicited by DSV4 are effective against multiple genotypes of the four DENV serotypes. Importantly, DSV4-elicited antibodies did not manifest enhancement potential in an in vivo ADE model. AG129 mice, which lack interferon α/β and γ receptors, are partially immune-compromised and sensitive to DENV infection, and in the presence of cross-reactive anti-dengue antibodies, manifest ADE, characterized by vascular leakage and pro-inflammatory cytokine production (Watanabe et al., 2015). Passive transfer of whole serum from DSV4-immunized BALB/c mice into AG129 mice suppressed viremia effectively and did not sensitize them to ADE upon sub-lethal infection with a challenge strain, DENV-2 S221. This was corroborated in a second approach by inoculation of pre-formed immune complexes (IC) generated in vitro by incubating DENV-2 S221 with DSV4-induced antibodies, into AG129 mice. While control AG129 mice, given ICs made using cross-reactive monoclonal and polyclonal antibodies, manifested aggressive ADE, ICs generated in vitro with anti-DSV4 antibodies did not result in ADE in vivo. Concomitantly, these mice did not manifest intestinal vascular leakage or pro-inflammatory cytokine elevation, in contrast to the control mice which received ICs made using homotypic polyclonal anti-DENV-2 antibodies. Further, another control group of AG129 mice that received free DENV-2 S221 (pre-incubated with normal mouse serum) began to show rapid mortality after 2 weeks, despite having very high serum nAb titers (serum dilution resulting in 50% in vitro DENV-2 neutralization was in the range of 3000–4000).

Anti-DSV4 antibodies elicited in Rhesus macaques, like their murine counterparts, neutralized all four DENVs in vitro. Macaques, which serve only as a DENV infection model, do not manifest any other aspect of dengue disease. Therefore, to assess if the macaque-induced anti-DSV4 antibodies possessed ADE capacity, whole serum from DSV4-immunized macaques was passively transferred into the AG129 mice. Challenging these mice with a sub-lethal dose of DENV-2 S221 did not escalate the DENV infection into a lethal one (Ramasamy et al., 2018). The lack of ADE is a critical attribute of DSV4 from the viewpoint of vaccine safety.

Currently, DSV4 vaccine development work is centered on process development and scale-up as a prelude to efficacy and toxicity studies in small animals before initiating cGMP production for clinical testing in the coming years.

Table 2
Comparison of LAV[a] and DSV4.

Vaccine characteristic	Tetravalent LAV	DSV4
Expression host	Mammalian cells	Yeast cells
Nature of immunogen	Mix of 4 monovalent LAVs	4-in-1 VLP
Immune response quality	Cross-reactive	Serotype-specific
Undesirable epitopes (prM, FL epitope of E, NS1[b])	Present	Absent
EDIII-directed nAb response	Low	High
Viral interference	Yes	No
Virus breathing	Yes	No
ADE potential (in vivo)	Yes[c]	Low/absent[d]

LAV, live attenuated vaccine; DSV4, tetravalent dengue subunit vaccine; VLP, virus-like particle; EDIII, E domain III; nAb, neutralizing antibody; ADE, antibody-dependent enhancement.

[a] The LAV could be any live attenuated virus vaccine (such as Dengvaxia, TetraVax-DV, TDV, etc.).

[b] Antibodies specific to all these epitopes are implicated in dengue pathogenesis.

[c] Based on the presence of enhancing epitopes in LAVs and Dengvaxia experience in humans (Halstead, 2017).

[d] Based on antibody-dependent enhancement experiments in AG129 mice (Ramasamy et al., 2018).

Box 1. Dengue vaccines: some key issues and questions.

What are the immune correlates of protection?
Flavivirus vaccines (such as those against yellow fever, Japanese encephalitis, and tick-borne encephalitis) elicit protection via nAbs. However, efficacy trials of Dengvaxia failed to establish nAb titers to protective efficacy for DENV-2. This has led to the perception that cellular immunity is (also) important.
Is it possible or necessary to design a vaccine effective against all structurally diverse forms and genotypes of all four serotypes?
Due to the ADE phenomenon, it is generally regarded that a dengue vaccine should elicit immunity to all four DENV serotypes. It is also considered that dengue vaccines must be effective against multiple genotypes of each serotype. The finding that DENVs exist in morphologically diverse forms and manifest 'breathing' has led to the suggestion that an effective vaccine must target this structural diversity as well.
Is it possible to protect dengue-naïve subjects against dengue by vaccination?
Dengvaxia efficacy trials suggested that prior serostatus is a determinant of vaccine efficacy. Subjects with prior DENV exposure were better-protected by the vaccine. However, an ideal dengue vaccine needs to be equally effective in both seronegative as well as seropositive recipients.
Can empirical re-formulation eliminate viral interference in tetravalent LAVs?
All current tetravalent LAV approaches are based on physical mixtures of empirically determined amounts of four monovalent vaccine viruses. Such mixtures manifest a tendency of viral interference, and could simulate a monotypic infection. In such a situation, seronegative recipients may be potentially sensitized to ADE later in life, upon natural DENV infection.
Can LAVs against DENV be safely deployed in regions with Zika virus prevalence?
Antibodies to the related flavivirus, Zika virus, can mediate ADE of DENVs. Due to this, the problem of viral interference of tetravalent dengue LAVs could be potentially exacerbated in Zika prevalent regions. Also, DENV antibodies can potentially cause ADE of Zika virus infection.
Is a recombinant subunit vaccine approach the answer to LAV problems?
Protein subunit vaccines circumvent viral interference as they are non-replicating. They can be designed to eliminate epitopes implicated in the induction of enhancing antibodies. They are unlikely to manifest 'breathing' behavior. However, the immune response elicited by protein subunit vaccines may not be as durable as that elicited by LAVs.
What is the utility of a controlled human infection model (CHIM) in dengue vaccine development?
There is no appropriate animal model of dengue infection to assess dengue vaccine candidates. There is increasing awareness about the potential utility of a dengue CHIM. This model may help define correlates of protection better and shortlist vaccine candidates for further clinical development. However, such a model is not yet scientifically validated. A critical limitation is that there is no way to assure that a subject participating in a CHIM study is not sensitized to ADE in the future, raising ethical concerns.
nAb, neutralizing antibody; DENV, dengue virus; ADE, antibody-dependent enhancement; LAV, live attenuated vaccine.

DSV4 differs from LAVs in many respects

DSV4 differs from LAVs in many potentially important respects (Table 2). DSV4 is made using the yeast *Pichia pastoris*, while the LAVs, such as Dengvaxia, TetraVax-DV, and TDV, are typically mammalian cell (Vero) culture-derived. In contrast to DSV4, which is based on a single tetravalent EDIII-based immunogen, the LAVs are typically physical mixtures of four monovalent attenuated viruses. As the EDIIIs are unique to each DENV serotype, DSV4 tends to elicit serotype-specific immune responses. In contrast, the LAVs, which carry all viral epitopes, elicit predominantly cross-reactive antibody responses, targeting the FL epitope on E domain II, as well as epitopes on prM and NS1. DENVs (and therefore DENV-derived LAVs) elicit only low levels of EDIII-directed serotype-specific nAbs. In contrast, DSV4 is designed to elicit predominantly EDIII-focused nAbs. There is a potential for viral interference in tetravalent LAVs among the four monovalent vaccine viruses, which could bias the immune response in favor of one serotype. DSV4 is a non-infectious and non-replicating vaccine and essentially circumvents the issue of viral interference. Additionally, the structural proteins of DENVs have been reported to display breathing behavior (Kuhn et al., 2015). Depending on temperature and time, the structural proteins on DENVs can undergo conformational changes that can transiently expose cryptic, potentially cross-reactive epitopes. It is likely that LAVs also possess this property. On the other hand, DSV4 consisting of serotype-specific epitopes displayed on a stable VLP scaffold, may offer a means to bypass 'breathing' associated with live viruses. DENVs (and by extension, LAVs) possess inherent capacity to induce predominantly cross-reactive antibodies. Such antibodies can potentially enhance DENV infection via the FcγR pathway. In contrast, DSV4, which elicits predominantly serotype-specific nAbs, has very low ADE potential.

Conclusions

A safe and effective dengue vaccine continues to be elusive. There are several unresolved questions and issues (see Box 1). It is becoming increasingly apparent that the quality of the immune response elicited by the vaccine is important. Current assays to measure vaccine performance have limitations. A recent study reports that DENV neutralization assays using culture-derived DENVs may overestimate nAb titers and, therefore, may not reflect vaccine efficacy reliably (Raut et al., 2019). As safety and efficacy of a dengue vaccine represent two sides of the same coin, the induction of nAbs without ADE is likely to be a more pragmatic correlate of protection until more precise correlates can be delineated. India is poised at the moment to test out two different dengue vaccine candidates in clinical trials in the near future, an LAV, TetraVax-DV, and a recombinant protein-based vaccine, DSV4.

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Authors' contributions

SS and NK: collation of information, analysis, interpretation, manuscript writing, and reading the final manuscript.

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Ethical approval

Approval was not required as the preparation of this article did not entail the use of animal or human subjects.

Conflict of interest

SS and NK are associated with the development of DSV4 vaccine.

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