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Live virus vaccines based on a yellow fever vaccine backbone: Standardized template with key considerations for a risk/benefit assessment☆,☆☆

Thomas P. Monath[a,1], Stephen J. Seligman[b,*], James S. Robertson[c,2], Bruno Guy[d], Edward B. Hayes[e,3], Richard C. Condit[f], Jean Louis Excler[g,h], Lisa Marie Mac[i], Baevin Carbery[i], Robert T. Chen[i], The Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG)[4]

[a] Kleiner Perkins Caufield & Byers, Menlo Park, CA 94025, USA
[b] Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595, USA
[c] National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, UK
[d] Discovery Department, Sanofi Pasteur, 69280 Marcy l'Etoile, France
[e] Barcelona Centre for International Health Research (CRESIB), 08036 Barcelona, Spain
[f] Department of Molecular Genetics & Microbiology, University of Florida, Gainesville, FL 32610, USA
[g] International AIDS Vaccine Initiative (IAVI), New York, NY 10004, USA
[h] U.S. Military HIV Research Program (MHRP), Bethesda, MD 20817, USA
[i] Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333, USA

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ABSTRACT

The Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG) was formed to evaluate the safety of live, recombinant viral vaccines incorporating genes from heterologous viruses inserted into the backbone of another virus (so-called "chimeric virus vaccines"). Many viral vector vaccines are in advanced clinical trials. The first such vaccine to be approved for marketing (to date in Australia, Thailand, Malaysia, and the Philippines) is a vaccine against the flavivirus, Japanese encephalitis (JE), which employs a licensed vaccine (yellow fever 17D) as a vector. In this vaccine, two envelope proteins (prM-E) of YF 17D virus were exchanged for the corresponding genes of JE virus, with additional attenuating mutations incorporated into the JE gene inserts. Similar vaccines have been constructed by inserting prM-E genes of dengue and West Nile into YF 17D virus and are in late stage clinical studies. The dengue vaccine is, however, more complex in that it requires a mixture of four live vectors each expressing one of the four dengue serotypes. This vaccine has been evaluated in multiple clinical trials. No significant safety concerns have been found. The Phase 3 trials met their endpoints in terms of overall reduction of confirmed dengue fever, and, most importantly a significant reduction in severe dengue and hospitalization due to dengue. However, based on results that have been published so far, efficacy in preventing serotype 2 infection is less than that for the other three serotypes. In the development of these chimeric vaccines, an important series of comparative studies of safety and efficacy were made using the parental YF 17D vaccine virus as a benchmark. In this paper, we use a standardized template describing the key characteristics of the novel flavivirus vaccine vectors, in comparison to the parental YF 17D vaccine. The template facilitates

☆ The findings, opinions, conclusions, and assertions contained in this consensus document are those of the individual members of the Working Group. They do not necessarily represent the official positions of each participant's organization (e.g., government, university, or corporations) and should not be construed to represent any Agency determination or policy.

* Corresponding author Tel.: +1 914 944 4647; fax: +1 914 923 8498.
E-mail addresses: stephen_seligman@nymc.edu, seligmanstephen@gmail.com (S.J. Seligman).
[1] Current affiliation: PaxVax Inc., Redwood City, CA 94063 and Hookipa Biotech AG, Vienna, Austria.
[2] Current affiliation: Retired.
[3] Deceased.
[4] See Acknowledgement for other V3SWG members.

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scientific discourse among key stakeholders by increasing the transparency and comparability of information. The Brighton Collaboration V3SWG template may also be useful as a guide to the evaluation of other recombinant viral vector vaccines.

1. Preamble

1.1. Need for working group and development of a standardized template for collection of key information for risk/benefit assessment of viral vector vaccines

Recombinant viral vectors provide an effective means for heterologous antigen expression in vivo and thus represent promising platforms for developing novel vaccines against human pathogens such as Ebola, human immunodeficiency virus (HIV), tuberculosis, and malaria) [1–9]. Preclinical evaluation of such viral vector vaccines has indicated their potential for immunization and an increasing number of candidate vaccines are entering human clinical trials. Improving our ability to anticipate potential safety issues and meaningfully assess or interpret safety data from trials of such new viral vector vaccines will increase the likelihood of public acceptance should they be licensed [10–13].

The Brighton Collaboration (www.brightoncollaboration.org) was formed in 2000 as an international voluntary collaboration to enhance the science of vaccine safety research [e.g., via development of standardized case definitions of adverse events following immunizations (AEFI)] [14]. In recognition of these needs in this domain, the Brighton Collaboration created the Viral Vector Vaccines Safety Working Group (V3SWG) in October 2008. Analogous to the value embodied in standardized case definitions for AEFI, the V3SWG believes a standardized template describing the key characteristics of a novel vaccine vector, when completed and maintained with the latest research, will facilitate scientific discourse among key stakeholders by increasing the transparency and comparability of information. Fortunately, the International AIDS Vaccine Initiative (IAVI) had already developed an internal tool to assess the risk/benefit of different viral vectors under its sponsorship. The IAVI graciously shared this tool with the V3SWG for adaptation and broader use as a standardized template for collection of key information for risk/benefit assessment on any viral vector vaccine. This tool was aimed at identifying potential major hurdles or concerns that would need to be addressed during the development of a vectored vaccine. The template collects information on the characteristics of the wild type virus from which the vector was derived as well as known effects of the proposed vaccine vector in animals and humans, manufacturing features, toxicology and potency, nonclinical studies, and human use, with an overall adverse effect and risk assessment.

The V3SWG hopes that eventually all developers/researchers of viral vector vaccines (especially those likely to be used in humans in the near future) will complete this template and submit it to the V3SWG and Brighton Collaboration for peer review and eventual publication in Vaccine. Following this, to promote transparency, the template will be posted and maintained on the Brighton Collaboration website for use/reference by various stakeholders. Furthermore, recognizing the rapid pace of new scientific developments in this domain (relative to AEFI case definitions), we hope to maintain these completed templates "wiki-" style with the help of Brighton Collaboration and each vector vaccine "community."

1.2. Need for risk/benefit assessment of live virus vaccines based upon a yellow fever vaccine backbone

Yellow fever (YF) is a mosquito-borne flavivirus disease that is has long endangered persons in sub-Saharan Africa and tropical areas in South America and is associated with a case fatality rate of 20-50% [15]. Since no effective anti-yellow fever virus medications are available and current mosquito-control measures are inadequate, vaccination remains paramount to YF prevention and control. Although appropriately controlled efficacy studies have never been carried out, the decline in YF cases following vaccination campaigns and the production in most studies of neutralizing antibodies in more than 95% of vaccinees are considered sufficient evidence that the 17D vaccine is effective. Regulations call for the vaccine to be administered every ten years; however, a recent recommendation by WHO's Strategic Advisory Group is that the vaccine need only be given once [16].

The live, attenuated YF 17D vaccine was previously deemed to be the world's safest and a model for the development of other live virus vaccines including polio, measles, mumps and varicella. Consequently, live vaccines against other flaviviruses, such as Japanese encephalitis virus, West Nile virus, and the four serotypes of dengue viruses, based on the YF 17D virus vaccine began to be developed. In 2001, however, severe rare reactions that were frequently fatal became recognized [17–19]. These reactions involved multiple organ systems and were named yellow fever vaccine-associated viscerotropic disease (YEL-AVD). As a consequence, in addition to live virus vaccines, inactivated vaccines, including one for YF, are being developed.

Risk groups for the development of YEL-AVD include elderly males as young as 56 years [20], women in their prime child-bearing years [21], and persons thymectomized as treatment for thymoma [22]. Guidelines for the definition of viscerotropic disease and for the association of YF vaccine with viscerotropic disease have been developed by a Brighton Collaboration working group [23]. In addition to YEL-AVD, other rare vaccine reactions include anaphylaxis and neurological disease called YF vaccine-associated neurological disease (YEL-AND). These reactions are rarely fatal or result in long term sequelae. Recognition of YEL-AVD has had a number of consequences including changes in recommendations for the vaccination of prospective travelers to and inhabitants in jungle (sylvatic) or savanna (intermediate) regions where they have a risk of exposure to YF virus-infected mosquitoes.

The feared complication of YF is spread of the virus to urban areas where the principal mosquito vector is *Aedes aegypti*, a mosquito that has become difficult if not impossible to eradicate in areas of huge tropical and subtropical municipalities with their large urban slums. Strategies for vaccination have also become complex as the number of cases of YEL-AVD in travelers have exceeded the number of cases of YF [15]. In South America, during periods of low virus activity, the risk to travelers of serious adverse events from the vaccine, particularly males over the age of 60, may be similar to that of developing yellow fever. In contrast, in Africa, the risk of acquiring yellow fever can be 600 times, and of death 700 times, the risk of vaccination.

An understanding of the makeup of the flavivirus genome is helpful in understanding the new vaccines being developed based upon the yellow fever vaccine virus as a vector. Flaviviruses are single stranded, positive sense RNA viruses. Their genomes encode three structural genes (capsid [C], pre-membrane [prM], envelope [E]) and at least seven non-structural genes (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) in that order [24]. The coding region is flanked by non-coding regions at the 5′ and 3′ ends of the genome. The YF 17D vaccine was developed by serial passage of a

Table 1
Risk/benefit assessment for live virus vaccines based on a yellow fever vaccine backbone.

1. Basic information	Information		
1.1. Author(s)	Thomas P. Monath MD/modified by Stephen J. Seligman MD/moderated by Jim S. Robertson MD		
1.2. Date completed/updated	March 1, 2011 (JSR), updated September 25, 2014 (SJS,TPM)		
2. Vaccine vector information	Information		
2.1. Name of vaccines	Yellow fever 17D-204, Yellow fever 17DD, ChimeriVax-TDV (CYD-TDV), ChimeriVax-JE, Imojev®, ChimeriVax WN		
2.2. Class/subtype	Flaviviridae/Flavivirus single strand positive-sense RNA virus		
2.3. Proposed route of administration	Subcutaneously		
3. Characteristics of wild type agent and attenuated vaccine derived from them	Information	Comments/concerns	Reference(s)
3.1. Disease(s) caused by wild type, the strength of evidence, severity, and duration of disease for the following categories:	Wild-type yellow fever (YF) virus causes hepatitis and hemorrhagic fever	Mortality rates of 20–50%	[15]
3.1a. Disease(s) caused by attenuated live yellow fever virus vaccine			
Overall	Yellow fever vaccine associated neurotropic (YEL-AND) and viscerotropic (YEL-AVD) serious adverse effects	Reporting rate of YEL-AND is 0.8 per 100,000 overall. According to VAERS data, reporting rate of YEL-AVD is 0.4 per 100,000 overall. Other estimates vary widely. Risk for YEL-AVD increases in males with age $\geq$56. In 2001, a series of reports appeared in Lancet describing severe, frequently fatal, viscerotropic reactions to yellow fever vaccine that stimulated surveillance of what had been considered the safest of live virus vaccines. Additional cases of YEL-AVD in prospective travelers and in inhabitants of South America were recognized. In S. America the incidence may be underestimated because much of the vaccine is administered to previously vaccinated individuals. In Peru the incidence of fatal reactions in a previously unimmunized population approached 1 in 10,000. In Africa surveillance for AEFI remains inadequate, although WHO has conducted follow-up for adverse events after mass YF vaccine campaigns, without definitive identification of YEL-AVD	[17–20,27–30]
In immunocompromized	Association of thymic disease with YEL-AVD. Immune deficiency a contraindication to YF 17D. Systemic lupus erythematosus on corticosteroids	Thymic disease is a contraindication to YF 17D. One report of YEL-AND (fatal) in a patient with HIV/AIDS 3 fatal cases. No safety issues after administration of YF17D in 102 HIV+ volunteers, although rate of serious adverse events of up to 3% cannot be excluded	[22,29,31–33]
In neonates, infants, children	Age < 6 months associated with risk of YEL-AND, and is a contraindication. Children aged 6–9 months only vaccinated under special circumstances and on the basis of current official advice		[15,34]
During pregnancy and in the unborn	Pregnancy a hypothetical contraindication. However congenital infections very rare and no reports of adverse effects on fetus	Two reports of neonates acquiring 17 D infection and YEL-AND via milk from recently vaccinated nursing mothers	[15,35]
Are there any other susceptible populations	Elderly have higher risk of YEL-AND and elderly males, YEL-AVD. Women of prime childbearing age	Reporting rate in persons >70 approx 2 per 100,000 for both YEL-AND and AVD	[20,21,27,36]
Animals	Wild-type YF causes similar disease in nonhuman primates (NHP), and (adapted strains) in hamsters, but is neurotropic (no hepatitis) in mice. YF 17D vaccine inoculated IC neurotropic/lethal in mice and causes self-limited encephalitis in monkeys	YF 17D vaccine is controlled by monkey neurovirulence test based on scoring inflammatory lesions in brain/spinal cord	[15]
3.2. Is there any known evidence of neurological or cardiac involvement of the wild type agent?	YEL-AND most often caused by neuroinvasion of CNS followed by acute meninogoencephalitis, generally self-limited, rare sequelae. Rare except in infants <6 mos. Wild-type YF does not cause encephalitis (though the virus is neurovirulent in mice and monkeys after intracerebral inoculation). Wild-type (Wt) virus and YEL-AVD associated with myocarditis, but not a prominent feature		

Table 1 (*Continued*)

1. Basic information	Information		
3.3. What is known about the types of human cells infected and the receptors used in humans and animals?	In humans and NHP, wild-type YF infects multiple types of lymphoid cells, including DCs and Kupffer cells, then spreads to hepatocytes. YF 17D infects skin at site of inoculation, regional nodes, then reticulo-endothelial system (RES) and bone marrow. Wt dengue virus infects a large variety of cell types, including monocytes, macrophages, DCs and possibly endothelial cells. Chimeric YF/DEN vaccines infect DCs in vitro		[37–39]
3.4. Does the agent replicate in the nucleus?	No		
3.5. What is the risk of integration into the human genome?	None		
3.6. Does the agent establish a latent or persistent infection?	Persistent YF 17D virus infection of experimentally infected monkeys reported. No evidence in wild-type yellow fever of humans, but not specifically studied. One report of RNA genomes of YF 17D in urine of humans approx. 6 mos. after vaccination. Chronic infection with West Nile, Japanese encephalitis, and TBE have been described in animal models and in humans	IgM antibody lasting up to 18 months following 17D vaccination in one study suggested the possibility of persistent infection	[40–43]
3.7. How does the wild type agent normally transmit?	By agency of blood feeding *Aedes* and *Haemagogus* spp. mosquitoes	YF 17D vaccine incapable of infecting mosquitoes. Recombinants produced by the insertion of dengue 4, JE and WN prM-E genes into wild-type YF showed a significant decrease in infectivity for mosquito vectors compared to wild-type YF	[15,44–49]
3.8. What is known about the mechanisms of immunity to the wild type agent?	Both wild-type and vaccine induced immunity against future or repeated infection principally via neutralizing antibodies. Innate immune responses play an important role in early defense. CTLs responsible for clearing infection and recovery	Defects in innate immunity (interferon pathways) may underlie susceptibility of rare individuals to severe vaccine associated SAEs (YEL-AVD). Interferon receptor k/o mice develop viscerotropic disease	[50–52]
3.9. Is there treatment required and readily available for the disease caused by the wild type agent?	No. see cited review		[53]
4. Characteristics of proposed chimeric vaccines	Information	Comments/concerns	Reference(s)
4.1. What is the basis of attenuation/inactivation?	Chimerization (replacement of structural membrane and envelope genes (prM-E) with genes from another flavivirus). Evidence that chimerization itself contributes to attenuation also comes from experiments showing that insertion of prM-E genes from wt DENV 4 in a wt YF Asibi backbone results in decreased virulence. Multiple mutations in the YF 17D non-structural genes that occurred during empirical passage to develop the attenuated 17D vaccine. In some vaccine constructs, mutations were also inserted in the prM-E genes of the gene donor virus to decrease neurovirulence (WN vaccine). For constructing the chimeric JE vaccine, prM-E genes from the attenuated SA1414.2 vaccine were used, already containing some mutations as compared to wt JE virus		[39,54–58]
4.2. What is the risk of reversion to virulence or recombination with wild type or other agents?	Negligible due to replacement of entire PrM-E gene (chimerization), redundant mutations. See discussion of recombination in reference cited	Attenuated YF 17D and 17DD vaccines differ from wild-type YF in the genes encoding 20 amino acids. In addition there are 4 nT changes in the 3′UTR that also might affect attenuation. The prM-E sequences in the YF vaccine result in 8 amino acid changes. Accordingly, the chimeric vaccines lack the mutations in the prM-E genes of 17D virus. Evaluation of chimeric vaccine candidates versus parental YF 17D with respect to markers of attenuation in vitro and in vivo have consistently shown that the chimeric viruses are more attenuated than the parent. More than 29,000 humans subjects have received ChimeriVax vaccines without significant safety issues. Empirical studies have been performed to assess the biological consequences of non-homologous recombination of the chimeric vaccine viruses with prM-E or backbone sequences from virulent flaviviruses. These studies have shown that such recombinational events do not result in restoration of a virulent phenotype or a virus that has increased potential to disseminate via mosquito bite	[44,56,59–63]

Table 1 (*Continued*)

1. Basic information	Information		
4.3 Are yellow fever virus and chimeric viruses derived from them genetically stable?	YF 17D vaccine is an uncloned mixture (genetic swarm) as shown for all other flaviviruses. The 17D vaccine contains multiple diverse plaque populations and subpopulations of virions that react with wild-type specific YF monoclonal antibodies. In one fatal case of encephalitis caused by YF 17D vaccine, the virus isolated from brain contained two mutations that were associated with increased neurovirulence. In contrast, 17D isolates from cases of YEL-AVD have not shown mutations or selection of a specific subpopulation or variant associated with these adverse events. Overall, sequencing studies show that yellow fever 17D vaccines as manufactured using the seed lot system, and the chimeric constructs derived from them, have demonstrated low rates of mutation during *in vitro* and *in vivo* replication. Furthermore, YF appears to be more genetically stable than other RNA viruses. This may be due in part to the high fidelity of its polymerase. However, 27% of the yellow fever fusion peptide sequences deposited in GenBank show changes in this highly conserved region in contrast to 5% of other pathogenic flaviviruses suggesting that yellow fever virus is not more genetically stable than other flaviviruses at least in this small region		[64–72]
4.3a. Are the chimeric vaccine candidates genetically stable during multiple passages?	Yes	All chimeric vaccine candidates have been assessed for genetic stability through serial passages. YF polymerase appears to be quite stable compared to other RNA viruses. ChimeriVax vaccines have all been made by molecular cloning and transfection of RNA into acceptable cell substrates to produce vaccine seed. The resulting seed stock is then biologically cloned (by plaque purification). Molecular and biological cloning promote genetic homogeneity of the vaccine virus. The actual vaccines are passed only a few times in cell cultures during manufacture. They are assessed for genetic stability on serial passage; after these viruses accumulate a few mutations related to adaptation to Vero cells, they have been quite stable on serial passages. Quality control procedures (sequencing) is used to monitor genetic stability of virus seeds and vaccine lots. Specifications for the consensus sequence are used for vaccine release	[39,57,68,70]
4.4. What is known about the genetic stability during in vivo replication?	Less empirical information, but no indication of instability, reversion to virulence	Serial in vivo passages in mice showed no reversion	[69]
4.5. Will a replication competent agent be formed?	The chimeric vaccines are replication competent		
4.6. What is the potential for shedding and transmission?	Considered unlikely, except for possible secretion in breast milk. The presence of virus titers as high as 6.2×10^9 genome equivalents/g in individuals with YEL-AVD increases the possibility of transmission	Chimeric YF/West Nile vaccine studied in horses; no evidence for shedding but vaccines intended for other species may need studies. Limited persistence and bio-distribution in monkeys after vaccination with Chimerivax-WN	[35,38,73,74]
4.7. Will the agent survive in the environment?	No		
4.8. Is there a non-human 'reservoir'?	Not for the attenuated vaccine virus		
4.9. Is there any evidence for or against safety during pregnancy?	See discussion of YF17D vaccine		
4.10. Can the vector accommodate multigenic inserts or will several vectors be required for multigenic vaccines?	See comments	Tetravalent YF/dengue vaccine required making 4 separate vectors and mixing in a formulation	[39,54]
4.11. What is known about the effect of pre-existing immunity on 'take', safety or efficacy in animal models?	No antivector immunity because prM-E (containing neutralizing epitopes) of YF replaced by corresponding genes of target vaccine virus. Prior YF immunity does not interfere. T cell responses to YF 17D non-structural proteins do not preclude effective immunization or re-use of vectors	Positive effect on chimeric dengue vaccine immunogenicity due to prior 17D immunity	See above reviews

Table 1 (*Continued*)

1. Basic information	Information		
5. Manufacturing	Information	Comments/Concerns	Reference(s)
5.1. Describe the source (e.g., isolation, synthesis)	Infectious cDNA clone of YF 17D vaccine virus Sanofi Pasteur – Swiftwater PA		
5.2. Describe the provenance of the vector including passage history and exposure to animal products	FDA licensed YF 17D vaccine	No fetal bovine serum (FBS) or animal products used in manufacturing process	
5.3. Can the vector be produced in an acceptable cell substrate?	Yes	Vero cells, WHO-87 cells, FDA bank deposited at ATCC	
5.4. Describe the proposed production process	prM-E of vector replaced with corresponding genes of virus against which immunity is desired	Chimeric RNA transfected into Vero cells to produce chimeric live attenuated vaccine	See above reviews
5.5. What are some Purity/Potential contaminants?	Virus purified from supernatant fluid by depth filtration, ultrafiltration, diafiltration		
Is there a large scale manufacturing feasibility?	Vaccines already made at commercial scale		
Are there any IP issues and is there free use of the vector?	Proprietary, vector system is covered by multiple patents	IP licensed by Acambis from St Louis University and NIH, and multiple new patents filed by Acambis. Acambis acquired by sanofi Pasteur in 2008	
6. Toxicology and potency (Pharmacology)	Information	Comments/Concerns	Reference(s)
6.1. What is known about the replication, transmission and pathogenicity in animals?	Multiple Good Laboratory Practice (GLP) toxicity studies performed in nonhuman primates (NHP) for chimeric vaccines against Japanese encephalitis, dengue, West Nile. The live chimeric viruses replicate in mice, hamsters, monkeys, cause transient viremia. Neurovirulence tests performed in infant mice and NHP, using licensed YF 17D vaccine as a reference control	In rodents and NHP, the chimeric vaccines were significantly less virulent than the licensed YF 17D vaccine	[55,57,75–77]
6.2. For replicating vectors, has a comparative virulence and viral kinetic study been conducted in permissive and susceptible species? (yes/no) If not, what species would be used for such a study? Is it feasible to conduct such a study?	Many such studies, see references		See above[54]
6.3. Does an animal model relevant to assess attenuation exist?	Reduced viremia in NHP infected with ChimeriVax-DEN versus Wt DEN viruses. For the encephalitides, e.g., JE and WN, rodents and NHP are excellent models in which to assess attenuation of neurovirulence. YF 17D is neurovirulent (and in infant mice neuroinvasive), whereas the chimeric vaccines are significantly attenuated. Specifications for release of seed viruses include demonstration of reduced neurovirulence in a GLP test in NHP	One of the difficulties in developing a dengue vaccine has been the absence of a convenient animal model. In nonhuman primates wild-type dengue causes a transient viremia, but no disease. A mouse model for dengue has been described, but since it involves immunocompromized mice, it can no be used to evaluate vaccine efficacy	See above [54,57,75,78]
6.4. Does an animal model for safety including immuno-compromised animals exist?	Unpublished studies in hamsters treated with cyclophosphamide showed no increase in virulence except for prolonged subclinical viremia. Chimeric JE vaccine was fully attenuated in type I/II IFN receptor KO mice (A129 or AG129)		[57,79]
6.5. Does an animal model for reproductive toxicity exist?	Yes, but not tested	Ongoing for chimeric dengue vaccine	
6.6. Does an animal model for immunogenicity and efficacy exists?	Hamsters, mice, horses, monkeys	See references and reviews	[80]
6.7. What is known about biodistribution?	Replicates in skin at site of inoculation, then draining nodes, then RES and bone marrow	Virus cleared after adaptive immunity established	[38]
6.8. Have neurovirulence studies been conducted?	Multiple	All lots of vaccine tested by sensitive neurovirulence test in infant mice. GLP neurovirulence studies in NHP for all new vaccine constructs, using licensed YF 17D as reference control	[75]
6.9. What is the evidence that the vaccines will generate a beneficial immune response in Rodent?	Rodents (mice, hamsters) immunized with single inoculation develop neutralizing antibodies and protected against challenge with wild-type virus corresponding to the inserted prM-E gene	Examples include Japanese encephalitis and West Nile	See above [54,57]

Table 1 (*Continued*)

1. Basic information	Information		
Non rodent?	Horses immunized and protected against West Nile	Licensed vaccine (Prevenile®, Intervet) for horses	[77,79,81–84]
NHP?	Multiple studies showing immunogenicity and protection against challenge with dengue, JE, WN		[54,77,81,83]
Human?	The ChimeriVax-JE vaccine (Imojev®) was approved in Australia, Thailand and other Asian countries based on Phase 1–3 studies showing non-inferiority of neutralizing antibody responses to approved JE vaccine. The ChimeriVax-WN vaccine has been shown to elicit protective levels of neutralizing antibodies as well as strong T cell responses in Phase 1–2 clinical trials. A placebo-controlled phase 2b study of tetravalent ChimeriVax-DEN in Thai children conducted in a single site demonstrated protection against types 1, 3 and 4 but, despite the production of neutralizing antibodies, there was no observed protection from disease with type 2 dengue, the most prevalent circulating serotype. A subsequent phase 3 study in Asian children conducted in 5 countries and 11 sites found 56% overall protection against dengue fever (which met the proscribed study end-point), however efficacy against dengue 2 was lower than for other serotype. 35% protection against type 2 dengue was observed, compared to 50 to 78% protection against the other serotypes. Vaccine efficacy of against dengue hemorrhagic fever (DHF) was 88.5% (per protocol) and 67.2% against hospitalization (intent to treat). A phase 3 study in Latin America further confirmed the results obtained in Asia, with a 61% overall protection against dengue fever, with an efficacy of 50% against serotype 1, 42% against serotype 2, 74% against serotype 3 and 77% against serotype 4. A significant protection was also observed against severe disease and hospitalization. Multiple previous Phase 2 studies also suggested a similar level of efficacy	The Thai study of ChimeriVax-DEN with confirmation from an Asian and Latin American study, raises the possibility that protective efficacy is lower against type 2 dengue. However, protection against hospitalization and severe dengue involved all 4 serotypes. In the phase 3 studies there were no deaths caused by dengue in the control or vaccine groups. Accordingly the efficacy of the vaccine in preventing mortality remains to be studied	[38,39,45,57,85–89] Sanofi Pasteur, unpublished data, [90]
6.10. Have challenge or efficacy studies been conducted with			
HIV?	See 3.1a in immunocompromized		
Other diseases?	JE, dengue, WN (see refs)		
7. Previous human use	Please type one of the following: yes, no, Unknown, N/A (non-applicable)	Comments	Reference(s)
7.1. Has the vector already been used for targeting the disease of vector origin (measles, BCG, rabies)?	Yes, licensed vaccine against YF		
7.2. What is known about the replication, transmission and pathogenicity of the vector in: healthy people? Immunocompromised? neonates, infants, children? pregnancy and in the unborn? gene therapy experiments? any other susceptible populations?	See first section		
7.3. Is there any previous human experience with a similar vector including in HIV+ (safety and immunogenicity records)?	non-applicable		
7.4. Is there any previous human experience with present vector including HIV+ (safety and immunogenicity records)?	see 3.1a		
7.5. What is known about the effect of pre-existing immunity on 'take', safety or efficacy in any human studies with this or different insert?	prior administration of 17D does not interfere with the chimeric vaccines		

Table 1 (*Continued*)

1. Basic information	Information			
7.6. Name some other non-HIV vaccines using same vector and describe some of the public health considerations.	none			
8. Overall Risk Assessment	Describe the toxicities	Please rate the risk as one of the following: none, minimal, low, moderate, high, or unknown	Comments	Reference(s)
8.1. What is the potential for causing serious unwanted effects and toxicities in:				
Healthy people?	Multiple trials of chimeric YF with JE, dengue, WN gene inserts have not shown any significant safety issues	The chimeric JE vaccine (ImoJev®) is licensed, the chimeric dengue vaccine is in Phase III and the WN vaccine is in Phase II. A veterinary vaccine against WN is licensed		See previous and [91]
Immunocompromised?	No data			
Neonates, infants, children?	Trials of the chimeric JE and dengue vaccines have been conducted in children 2 years and older, and have shown them to be safe and well tolerated.			
Pregnancy and in the unborn?	No data. Parental YF 17D (and therefore ChimeriVax) not be administered during pregnancy unless clearly required, based on a high risk exists of natural infection			
Other susceptible populations?				
8.2. What is the risk of neurotoxicity/neuroinvasion or cardiac effects?	The vaccines are significantly less neurovirulent than the licensed YF 17D vaccine			[75]
8.3. What is the potential for shedding and transmission in risk groups?	YF 17D rarely transmitted to infants via breast milk Viremia titers as high as 6.2×10^9 genome equivalents/g in individuals dying with YEL-AVD raise the possibility of transmission by blood but likelihood of adverse event low since the virus in such individuals has few/no mutations	However, multiple studies have shown that the chimeric vaccines are not infectious for mosquitoes		[45–49,74]
8.4. What is the risk of adventitious agent (including TSE) contamination?	Minimal. Seed viruses and each vaccine lot are tested for adventitious viruses. No animal products used in manufacturing.			
8.5. Can the vector be manufactured at scale in an acceptable process?	Yes, the JE vaccine is at commercial scale now; Sanofi Pasteur has constructed a commercial factory for the dengue vaccine	10,000–50,000 doses per L–very efficient process		
8.6. Can virulence, attenuation and toxicity be adequately assessed in preclinical models?	Yes			
8.7. Rate the evidence that a beneficial response will be obtained in humans.	Already known—neutralizing antibodies in >95% of human subjects. T cell responses to the E protein conserved in humans	The dengue vaccine contains a mixture of 4 separate viruses each representing 1 serotype. Interference between the individual viruses is observed, so that 2-3 doses are required for complete immunization. In contrast the JE and WN vaccines are monovalent and a single dose provides durable immunity and T cell memory. The proposed correlate of protection for chimeric vaccines against encephalitic viruses (JE, WN) is the level of neutralizing antibodies (PRNT50 titer), and a level of >10 is considered protective; the chimeric vaccines elicit neutralizing antibody titers in great excess over this minimum protective level. However, an immune correlate is not established yet for dengue vaccines. Cellular immunity may also contribute to protection, and it has been shown that chimeric vaccines are able to induce significant responses in this respect.		[39,57,91–93]
9. Adverse Effect Assessment	Describe the adverse effects	Please rate the risk as one of the following: none, minimal, low, moderate, high, or unknown	Comments	Reference(s)
9.1. Describe the adverse effects observed				
Mild local reactions	Erythema, pain	Minimal		
Mild systematic reactions	Similar to placebo			
Moderate local reactions	Erythema, pain	Minimal		
Moderate systematic reactions	Similar to placebo			
Severe local reactions		None		
Severe systematic reactions		None		
10. Administration Assessment	Information	Comments/Concerns	Reference(s)	

Table 1 (*Continued*)

1. Basic information	Information
10.1. What is the average Tissue Culture Infections Dose per millimeter (TCID/ml)?	Human dose is 3-5 log10 plaque forming units (PFU).
10.2. What is the highest TCID/ml that can be used before cell toxicity?	The viruses cause CPE in vitro at low multiplicities of infection (MOI).
10.3. Are different demographics affected differently?	No information
References	Information

wild-type virus in chicken embryo cultures resulting in the acquisition of nucleotide mutations some of which do not cause changes in the translated amino acids (synonymous) and some of which do (non-synonymous). In the YF-17D vectored vaccines that are being developed for Japanese encephalitis, West Nile and the four serotypes of dengue viruses, the coding regions of the prM-E proteins of the corresponding viruses are substituted for those in the YF 17D virus vaccine, resulting in a chimeric virus. The YF-17D viral genome excluding the prM-E genes is referred to as the "backbone". Although the production of such chimeric viruses alone may decrease their virulence, preclinical studies have demonstrated that the YF backbone has sufficient mutations by itself to maintain vaccine attenuation of chimeric vaccines bearing the dengue surface antigens [22]. Another aspect of the chimeric flavivirus technology that is distinguished from other viral vectors, is that anti-vector immunity is not a significant problem for flavivirus vaccine development. This feature is due to the fact that the prM-E region is solely responsible for generating epitopes recognized by neutralizing antibodies. Thus, the chimeric vector contains the only neutralizing antigens of the intended target for immunization. Previous immunization with YF17D and T cell responses to the YF 17D backbone are insufficient to prevent effective immunization with a chimeric vector expressing a heterologous flavivirus prM-E transgene.

The efforts of the V3SWG were focused initially on the above flavivirus vaccines. Not addressed are additional possible vaccines in which nucleotide sequences encoding for epitopes of other microorganisms might be added into the complete YF virus vaccine yielding live virus vaccines with additional vaccination potential. Vaccines employing other methods of attenuating flaviviruses through mechanisms such as nucleotide deletions and which do not involve a viral vector are not considered in this project [25].

The chimeric vaccines indicated for the prevention of dengue, Japanese encephalitis, and West Nile will be considered new entities from the regulatory perspective, and will need to be independently assessed for safety and efficacy. However, in the development of these new vaccines, the parental YF 17D vaccine virus has provided an important comparator and benchmark in all preclinical and many clinical trials. For example, the monkey neurovirulence test is an important measure of safety of YF 17D vaccines, and YF 17D was used as the reference strain in many studies, which showed that the chimeric vaccines were more attenuated than parental YF 17D. An important regulatory question thus arises as to whether the age range for vaccination, precautions and contraindications in labelling for use of YF 17D vaccines should apply to the new, chimeric vaccines. This question is particularly important since data on very rare adverse events will likely not be available at the time the new vaccines are approved. The template supplied in this paper contains information that can potentially be useful in considering how new chimeric vaccines should be described in reference to parental YF 17D vaccines.

1.3. Methods for developing, completing and reviewing the standardized template

Following the process described in the accompanying overview paper [26] as well as on the Brighton Collaboration website (http://cms.brightoncollaboration.org:8080/public/what-we-do/setting-standards/case-definitions/process.html), the Brighton Collaboration V3SWG was formed in October 2008 and includes ~15 members with clinical, academic, public health, regulatory and industry backgrounds with appropriate expertise and interest. The composition of the working and reference group as well as results of the web-based survey completed by the reference group with subsequent discussions in the working group can be viewed at http://www.brightoncollaboration.org/internet/en/index/workinggroups.html. The workgroup meets via emails and monthly conference calls coordinated by a secretariat [currently at CDC's Division of HIV/AIDS Prevention].

The V3SWG invited a flavivirus expert, Thomas P. Monath (TPM), who has been intimately associated with the development of flavivirus vaccines based on the YF virus vaccine backbone to complete the template in 2011. The first draft was then critiqued by a member of the working group knowledgeable about flaviviruses, Stephen J. Seligman (SJS), moderated by another member, James S. Robertson (JSR), discussed by the V3SWG as a whole, and then peer reviewed by reference groups (e.g., American Society of Virology, American Society of Tropical Medicine and Hygiene) and the Brighton Collaboration membership. Bruno Guy updated the template with new information as of October, 2014. Sections 8 (overall risk assessment) and 9 (adverse effect assessment) of the template seeks to rate the risk of the viral vector in various situations as: none, minimal, low, moderate, high, or unknown. An initial assessment was made by TPM and then reviewed by others, based largely on the anticipated frequency and severity of the vaccine adverse event versus the expected frequency and severity of the target vaccine preventable disease [15]. Depending on the season, the risk of the yellow fever vaccine approaches the risk of YF in S. America. In Africa the risk of yellow fever is usually much greater than the risk of the vaccine. The V3SWG may develop more explicit criteria for standardizing the rating of these risks in the future with further experience.

The resulting template is submitted as a guideline for evaluating the current issues in development of vaccines based on the yellow fever virus vaccine backbone.

2. Standardized template (Table 1)

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.10.004.

References

[1] Liniger M, Zuniga A, Naim HY. Use of viral vectors for the development of vaccines. Expert Rev Vaccines 2007;6(2):255–66.
[2] Excler JL, Parks CL, Ackland J, Rees H, Gust ID, Koff WC. Replicating viral vectors as HIV vaccines: summary report from the IAVI-sponsored satellite symposium at the AIDS vaccine 2009 conference. Biologicals 2010;38(4):511–21.
[3] Limbach KJ, Richie TL. Viral vectors in malaria vaccine development. Parasite Immunol 2009;31(9):501–19.
[4] Xing Z, Lichty BD. Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. Tuberculosis (Edinb) 2006;86(3–4):211–7.
[5] Naim HY. Applications and challenges of multivalent recombinant vaccines. Hum Vaccin Immunother 2012;9(3).
[6] Johnson JA, Barouch DH, Baden LR. Nonreplicating vectors in HIV vaccines. Curr Opin HIV AIDS 2013;8(5):412–20.
[7] Parks CL, Picker LJ, King CR. Development of replication-competent viral vectors for HIV vaccine delivery. Curr Opin HIV AIDS 2013;8(5):402–11.
[8] Wong G, Audet J, Fernando L, Fausther-Bovendo H, Alimonti JB, Kobinger GP, et al. Immunization with vesicular stomatitis virus vaccine expressing the Ebola glycoprotein provides sustained long-term protection in rodents. Vaccine 2014;32(43):5722–9.
[9] Mullard A. Experimental Ebola drugs enter the limelight. Lancet 2014;384(9944):649.
[10] WHO Initiative for Vaccine Research. WHO informal consultation on characterization and quality aspect of vaccines based on live viral vectors December 2003. Accessed from: http://www.who.int/vaccine_research/documents/vvreport/en/index1.html on 30 October 2014.
[11] Amarasinghe A, Black S, Bonhoeffer J, Carvalho SM, Dodoo A, Eskola J, et al. Effective vaccine safety systems in all countries: a challenge for more equitable access to immunization. Vaccine 2013;31(Suppl 2):B108–14.
[12] MacDonald NE, Smith J, Appleton M. Risk perception, risk management and safety assessment: what can governments do to increase public confidence in their vaccine system? Biologicals 2012;40(5):384–8.
[13] Chen RT, Hibbs B. Vaccine safety: current and future challenges. Pediatr Ann 1998;27(7):445–55.
[14] Kohl KS, Bonhoeffer J, Chen R, Duclos P, Heijbel H, Heininger U, et al. The Brighton Collaboration: enhancing comparability of vaccine safety data. Pharmacoepidemiol Drug Saf 2003;12(4):335–40.
[15] Monath TP, GM, Staples JE, Barrett AD. Yellow fever vaccine. In: Plotkin SA, Orenstein WA, O PA, editors. Vaccines. 6th ed. China: Saunders; 2012. p. 870–968.
[16] Meeting of the Strategic Advisory Group of Experts on immunization, April 2013 - conclusions and, recommendations., Wkly Epidemiol. Rec 2013;88(20):201–6.
[17] Vasconcelos PF, Luna EJ, Galler R, Silva LJ, Coimbra TL, Barros VL, et al. Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. Lancet 2001;358(9276):91–7.
[18] Martin M, Tsai TF, Cropp B, Chang GJ, Holmes DA, Tseng J, et al. Fever and multisystem organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. Lancet 2001;358(9276):98–104.
[19] Chan RC, Penney DJ, Little D, Carter IW, Roberts JA, Rawlinson WD. Hepatitis and death following vaccination with 17D-204 yellow fever vaccine. Lancet 2001;358(9276):121–2.
[20] Seligman SJ. Risk groups for yellow fever vaccine-associated viscerotropic disease (YEL-AVD). Vaccine 2014;32:5769–75.
[21] Seligman SJ. Yellow fever virus vaccine-associated deaths in young women. Emerg Infect Dis 2011;17(10):1891–3.
[22] Barwick Eidex R, Yellow Fever Vaccine Safety Working G. History of thymoma and yellow fever vaccination. Lancet 2004;364(9438):936.
[23] Gershman MD, Staples JE, Bentsi-Enchill AD, Breugelmans JG, Brito GS, Camacho LA, et al. Viscerotropic disease: case definition and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine 2012;30(33):5038–58.
[24] Rice CM, Lenches EM, Eddy SR, Shin SJ, Sheets RL, Strauss JH. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. Science 1985;229(4715):726–33.
[25] Durbin AP, Kirkpatrick BD, Pierce KK, Elwood D, Larsson CJ, Lindow JC, et al. A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive adults: a randomized, double-blind clinical trial. J Infect Dis 2013;207(6):957–65.
[26] Chen RT, Carbery B, Mac L, Berns KI, Chapman L, Condit RC, et al. The Brighton Collaboration Viral Vector Vaccine Safety Working Group (V3SWG). Vaccine. 2014 In press, Doi: 10.1016/j.vaccine.2014.09.035.
[27] Lindsey NP, Schroeder BA, Miller ER, Braun MM, Hinckley AF, Marano N, et al. Adverse event reports following yellow fever vaccination. Vaccine 2008;26(48):6077–82.
[28] Cottin P, Niedrig M, Domingo C. Safety profile of the yellow fever vaccine Stamaril(R): a 17-year review. Expert Rev Vaccines 2013;12(11):1351–68.
[29] Whittembury A, Ramirez G, Hernandez H, Ropero AM, Waterman S, Ticona M, et al. Viscerotropic disease following yellow fever vaccination in Peru. Vaccine 2009;27(43):5974–81.
[30] Breugelmans JG, Lewis RF, Agbenu E, Veit O, Jackson D, Domingo C, et al. Adverse events following yellow fever preventive vaccination campaigns in eight African countries from 2007 to 2010. Vaccine 2013;31(14):1819–29.
[31] Kengsakul K, Sathirapongsasuti K, Punyagupta S. Fatal myeloencephalitis following yellow fever vaccination in a case with HIV infection. J Med Assoc Thai 2002;85(1):131–4.
[32] Martins RMMM, Santos EM, Cruz RLS, Santos PG, e.a. Carvalho SMD. Yellow fever vaccine post-marketing surveillance in Brazil. Procedia Vaccinol 2010;(2):178–83.
[33] Veit O, Niedrig M, Chapuis-Taillard C, Cavassini M, Mossdorf E, Schmid P, et al. Immunogenicity and safety of yellow fever vaccination for 102 HIV-infected patients. Clin Infect Dis 2009;48(5):659–66.
[34] Committee for Medicinal Products for Human Use (CHMP). Summary information on referral opinion pursuant to article 30 of council directive 2001/83/EC for stamaril and associated names. London: Committee for Medicinal Products for Human Use (CHMP); 2006. p. 1 (See Annex I).
[35] Centers for Disease, Control and Prevention. Transmission of yellow fever vaccine virus through breast-feeding—Brazil, 2009. MMWR Morb Mortal Wkly Rep 2010;59(5):130–2.
[36] Khromava AY, Eidex RB, Weld LH, Kohl KS, Bradshaw RD, Chen RT, et al. Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine 2005;23(25):3256–63.
[37] Monath TP, Barrett AD. Pathogenesis and pathophysiology of yellow fever. Adv Virus Res 2003;60:343–95.
[38] Monath TP, Liu J, Kanesa-Thasan N, Myers GA, Nichols R, Deary A, et al. A live, attenuated recombinant West Nile virus vaccine. Proc Natl Acad Sci U S A 2006;103(17):6694–9.
[39] Guy B, Guirakhoo F, Barban V, Higgs S, Monath TP, Lang J. Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue: West Nile and Japanese encephalitis viruses. Vaccine 2010;28(3):632–49.
[40] Monath TP. Neutralizing antibody responses in the major immunoglobulin classes to yellow fever 17D vaccination of humans. Am J Epidemiol 1971;93(2):122–9.
[41] Murray K, Walker C, Herrington E, Lewis JA, McCormick J, Beasley DW, et al. Persistent infection with West Nile virus years after initial infection. J Infect Dis 2010;201(1):2–4.
[42] Gritsun TS, Frolova TV, Zhankov AI, Armesto M, Turner SL, Frolova MP, et al. Characterization of a siberian virus isolated from a patient with progressive chronic tick-borne encephalitis. J Virol 2003;77(1):25–36.
[43] Domingo C, Yactayo S, Agbenu E, Demanou M, Schulz AR, Daskalow K, et al. Detection of yellow fever 17D genome in urine. J Clin Microbiol 2011;49(2):760–2.
[44] McGee CE, Tsetsarkin K, Vanlandingham DL, McElroy KL, Lang J, Guy B, 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 2008;197(5):686–92.
[45] Reid M, Mackenzie D, Baron A, Lehmann N, Lowry K, Aaskov J, et al. Experimental infection of Culex annulirostris. Culex gelidus, and Aedes vigilax with a yellow fever/Japanese encephalitis virus vaccine chimera (ChimeriVax-JE) Am J Trop Med Hyg 2006;75(4):659–63.
[46] Bhatt TR, Crabtree MB, Guirakhoo F, Monath TP, Miller BR. Growth characteristics of the chimeric Japanese encephalitis virus vaccine candidate, ChimeriVax-JE (YF/JE SA14–14–2), in Culex tritaeniorhynchus, Aedes albopictus, and Aedes aegypti mosquitoes. Am J Trop Med Hyg 2000;62(4):480–4.
[47] Johnson BW, Chambers TV, Crabtree MB, Guirakhoo F, Monath TP, Miller BR. 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 2004;70(1):89–97.
[48] Higgs S, Vanlandingham DL, Klingler KA, McElroy KL, McGee CE, Harrington L, et al. Growth characteristics of ChimeriVax-Den vaccine viruses in Aedes aegypti and Aedes albopictus from Thailand. Am J Trop Med Hyg 2006;75(5):986–93.
[49] Kazimirova M, Mantel N, Raynaud S, Slovak M, Ustanikova K, Lang J, et al. Evaluation of chimeric yellow fever 17D/dengue viral replication in ticks. Vector Borne Zoonotic Dis 2012;12(11):979–85.
[50] Mason RA, Tauraso NM, Spertzel RO, Ginn RK. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. Appl Microbiol 1973;25(4):539–44.
[51] Pulendran B, Miller J, Querec TD, Akondy R, Moseley N, Laur O, et al. Case of yellow fever vaccine–associated viscerotropic disease with prolonged viremia, robust adaptive immune responses, and polymorphisms in CCR5 and RANTES genes. J Infect Dis 2008;198(4):500–7.

[52] Pulendran B. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. Nat Rev Immunol 2009;9(10):741–7.
[53] Monath TP. Treatment of yellow fever. Antiviral Res 2008;78(1):116–24.
[54] Monath TP. Recombinant, chimeric, live, attenuated vaccines against Flaviviruses and Alphaviruses. In: RRDPM C, editor. Replicating vaccines: a new generation. Basel: Burkhäuser Verlag; 2010. p. 349–438.
[55] Arroyo J, Guirakhoo F, Fenner S, Zhang ZX, Monath TP, Chambers TJ. Molecular basis for attenuation of neurovirulence of a yellow fever Virus/Japanese encephalitis virus chimera vaccine (ChimeriVax-JE). J Virol 2001;75(2):934–42.
[56] McGee CE, Lewis MG, Claire MS, Wagner W, Lang J, Guy B, 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 2008;197(5):693–7.
[57] Dayan GH, Pugachev K, Bevilacqua J, Lang J, Monath TP. Preclinical and clinical development of a YFV 17 D-based chimeric vaccine against West Nile virus. Viruses 2013;5(12):3048–70.
[58] Xie H, Cass AR, Barrett AD. Yellow fever 17D vaccine virus isolated from healthy vaccinees accumulates very few mutations. Virus Res 1998;55(1):93–9.
[59] Seligman SJ, Gould EA. Live flavivirus vaccines: reasons for caution. Lancet 2004;363(9426):2073–5.
[60] Monath TP, Kanesa-Thasan N, Guirakhoo F, Pugachev K, Almond J, Lang J, et al. Recombination and flavivirus vaccines: a commentary. Vaccine 2005;23(23):2956–8.
[61] McGee CE, Tsetsarkin KA, Guy B, Lang J, Plante K, Vanlandingham DL, et al. Stability of yellow fever virus under recombinatory pressure as compared with chikungunya virus. PLoS One 2011;6(8):e23247.
[62] Taucher C, Berger A, Mandl CW. A trans-complementing recombination trap demonstrates a low propensity of flaviviruses for intermolecular recombination. J Virol 2010;84(1):599–611.
[63] Pugachev KV, Schwaiger J, Brown N, Zhang ZX, Catalan J, Mitchell FS, et al. Construction and biological characterization of artificial recombinants between a wild type flavivirus (Kunjin) and a live chimeric flavivirus vaccine (ChimeriVax-JE). Vaccine 2007;25(37–38):6661–71.
[64] Pugachev KV, Ocran SW, Guirakhoo F, Furby D, Monath TP. Heterogeneous nature of the genome of the ARILVAX yellow fever 17D vaccine revealed by consensus sequencing. Vaccine 2002;20(7–8):996–9.
[65] Liprandi F. Isolation of plaque variants differing in virulence from the 17D strain of yellow fever virus. J Gen Virol 1981;56(Pt 2):363–70.
[66] Jennings AD, Gibson CA, Miller BR, Mathews JH, Mitchell CJ, Roehrig JT, et al. Analysis of a yellow fever virus isolated from a fatal case of vaccine-associated human encephalitis. J Infect Dis 1994;169(3):512–8.
[67] Galler R, Pugachev KV, Santos CL, Ocran SW, Jabor AV, Rodrigues SG, et al. Phenotypic and molecular analyses of yellow fever 17DD vaccine viruses associated with serious adverse events in Brazil. Virology 2001;290(2):309–19.
[68] Mantel N, Girerd Y, Geny C, Bernard I, Pontvianne J, Lang J, et al. Genetic stability of a dengue vaccine based on chimeric yellow fever/dengue viruses. Vaccine 2011;29(38):6629–35.
[69] Guirakhoo F, Zhang ZX, Chambers TJ, Delagrave S, Arroyo J, Barrett AD, et al. Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. Virology 1999;257(2):363–72.
[70] Pugachev KV, Guirakhoo F, Ocran SW, Mitchell F, Parsons M, Penal C, et al. High fidelity of yellow fever virus RNA polymerase. J Virol 2004;78(2):1032–8.
[71] Barban V, Girerd Y, Aguirre M, Gulia S, Petiard F, Riou P, et al. High stability of yellow fever 17D-204 vaccine: a 12-year restrospective analysis of large-scale production. Vaccine 2007;25(15):2941–50.
[72] Seligman SJ. Constancy and diversity in the flavivirus fusion peptide. Virol J 2008;5:27.
[73] Long MT, Gibbs EP, Mellencamp MW, Zhang S, Barnett DC, Seino KK, et al. Safety of an attenuated West Nile virus vaccine, live Flavivirus chimera in horses. Equine Vet J 2007;39(6):486–90.
[74] Doblas A, Domingo C, Bae HG, Bohorquez CL, de Ory F, Niedrig M, et al. Yellow fever vaccine-associated viscerotropic disease and death in Spain. J Clin Virol 2006;36(2):156–8.
[75] Monath TP, Myers GA, Beck RA, Knauber M, Scappaticci K, Pullano T, et al. Safety testing for neurovirulence of novel live, attenuated flavivirus vaccines: infant mice provide an accurate surrogate for the test in monkeys. Biologicals 2005;33(3):131–44.
[76] Arroyo J, Miller C, Catalan J, Myers GA, Ratterree MS, Trent DW, et al. ChimeriVax-West Nile virus live-attenuated vaccine: preclinical evaluation of safety, immunogenicity, and efficacy. J Virol 2004;78(22):12497–507.
[77] Monath TP, Levenbook I, Soike K, Zhang ZX, Ratterree M, Draper K, et al. Chimeric yellow fever virus 17D-Japanese encephalitis virus vaccine: dose-response effectiveness and extended safety testing in rhesus monkeys. J Virol 2000;74(4):1742–51.
[78] Bente DA, Melkus MW, Garcia JV, Rico-Hesse R. Dengue fever in humanized NOD/SCID mice. J Virol 2005;79(21):13797–9.
[79] Lobigs M, Larena M, Alsharifi M, Lee E, Pavy M. Live chimeric and inactivated Japanese encephalitis virus vaccines differ in their cross-protective values against Murray Valley encephalitis virus. J Virol 2009;83(6):2436–45.
[80] Tesh RB, Arroyo J, Travassos Da Rosa AP, Guzman H, Xiao SY, Monath TP. Efficacy of killed virus vaccine, live attenuated chimeric virus vaccine, and passive immunization for prevention of West Nile virus encephalitis in hamster model. Emerg Infect Dis 2002;8(12):1392–7.
[81] Guirakhoo F, Pugachev K, Zhang Z, Myers G, Levenbook I, Draper K, et al. Safety and efficacy of chimeric yellow Fever-dengue virus tetravalent vaccine formulations in nonhuman primates. J Virol 2004;78(9):4761–75.
[82] Guirakhoo F, Arroyo J, Pugachev KV, Miller C, Zhang ZX, Weltzin R, et al. Construction, safety, and immunogenicity in nonhuman primates of a chimeric yellow fever-dengue virus tetravalent vaccine. J Virol 2001;75(16):7290–304.
[83] Monath TP, Soike K, Levenbook I, Zhang ZX, Arroyo J, Delagrave S, et al. Recombinant, chimaeric live, attenuated vaccine (ChimeriVax) incorporating the envelope genes of Japanese encephalitis (SA14-14-2) virus and the capsid and nonstructural genes of yellow fever (17D) virus is safe, immunogenic and protective in non-human primates. Vaccine 1999;17(15–16):1869–82.
[84] Guirakhoo F, Pugachev K, Arroyo J, Miller C, Zhang ZX, Weltzin R, et al. Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine: genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. Virology 2002;298(1):146–59.
[85] Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet 2012;380(9853):1559–67.
[86] Monath TP, McCarthy K, Bedford P, Johnson CT, Nichols R, Yoksan S, et al. Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections. Vaccine 2002;20(7-8):1004–18.
[87] Monath TP, Guirakhoo F, Nichols R, Yoksan S, Schrader R, Murphy C, et al. Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese encephalitis antigen. J Infect Dis 2003;188(8):1213–30.
[88] Morrison D, Legg TJ, Billings CW, Forrat R, Yoksan S, Lang J. A novel tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in flavivirus-naive adults. J Infect Dis 2010;201(3):370–7.
[89] Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014;384(9951):1358–65.
[90] da Costa VG, Marques-Silva AC, Floriano VG, Moreli ML. Safety, immunogenicity and efficacy of a recombinant tetravalent dengue vaccine: A meta-analysis of randomized trials. Vaccine 2014;32(39):4885–92.
[91] Torresi J, McCarthy K, Feroldi E, Meric C. Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: Randomised controlled phase 3 trials. Vaccine 2010;28(50):7993–8000.
[92] Guy B, Nougarede N, Begue S, Sanchez V, Souag N, Carre M, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine 2008;26(45):5712–21.
[93] Smith HL, Monath TP, Pazoles P, Rothman AL, Casey DM, Terajima M, et al. Development of antigen-specific memory CD8+ T cells following live-attenuated chimeric West Nile virus vaccination. J Infect Dis 2011;203(4):513–22.