The construction of similar YF 17D/DEN2 and YF 17D/DEN1 chimeras has been reported by two other groups (98,103–105). The induction of a robust $CD8^+$ T-cell response against the DEN2-specific prM-E envelope proteins was observed in mice (103), and Galler's group has demonstrated good safety and immunogenicity of their chimeras in monkeys, tested monovalently (104,105).

As was the case for ChimeriVax-JE, there is very little potential for transmission of ChimeriVax-DEN vaccine viruses by mosquitoes. *A. aegypti*, the principal DEN and YF virus vector, and *A. albopictus* mosquitoes were fed on artificial blood meals containing each of the viruses or a mixture of all four viruses. In contrast to wild-type DEN, the vaccine viruses were invariably highly attenuated with respect to their ability to infect mosquitoes and particularly with respect to dissemination from the gut to the salivary glands (106–108).

An initial phase I clinical trial of the ChimeriVax-DEN2 vaccine candidate was conducted to evaluate its safety, tolerability, and immunogenicity in healthy adults with and without prior YF vaccination ($n = 14$ per study group) (109). The vaccine was well tolerated. Most mild adverse events in the high (5 $\log_{10}$ pfu) and low (3 $\log_{10}$ pfu) dose groups were similar to a control YF-VAX group (5 $\log_{10}$ pfu of YF 17D), and there were no serious side effects. Mean peak viremias in all groups were below 2 $\log_{10}$ pfu/mL. 100% and 92.3% of subjects in the high and low ChimeriVax-DEN2 groups, respectively, seroconverted to DEN2, and 92% of subjects inoculated with YF-VAX seroconverted to YF 17D virus. High serum titers of DEN2 neutralizing antibodies were induced by day 31 ($\sim$1:350) and remained similarly high at 6 and 12 months post-immunization with ChimeriVax-DEN2, demonstrating excellent durability of the immune response. Pre-immunity to YF did not interfere with ChimeriVax-DEN2 immunization. T-cell responses against inactivated ChimeriVax-DEN2 antigen were detected using IFNγ ELISA in the majority of ChimeriVax-DEN2 immunized subjects. Interestingly, YF-immune subjects inoculated with ChimeriVax-DEN2 (but not YF naïve subjects) seroconverted to all four DEN serotypes. Although the underlying immunological mechanism of this phenomenon needs to be further investigated, this finding may have important practical implications for the development of tetravalent DEN vaccine.

Several tetravalent formulations are currently being tested by Sanofi Pasteur in phase I/II clinical trials, and results will be available shortly.

ChimeriVax-WN

ChimeriVax-WN was constructed using the prM-E genes from the New York-1999 WN strain. The chimera, referred to as ChimeriVax-WN01, was recovered following transfection of Vero cells and replicated to titers in excess of 7 $\log_{10}$ pfu/mL. This chimera was found to be significantly attenuated for mice when compared with both its WN parent and YF 17D. It was not neuroinvasive, but retained a degree of residual neurovirulence (110). To obtain a more attenuated vaccine candidate for human use, three attenuating SA14-14-2-specific amino acid changes were introduced into the E protein of ChimeriVax-WN01 at residues 107, 316, and 440, resulting in ChimeriVax-WN02 variant. The latter was completely avirulent in adult mice, and dramatically less neurovirulent than YF 17D in suckling mice ($p < 0.0001$) (110). Neuropathological scores after IC inoculation of both rhesus and cynomolgus monkeys were lower for ChimeriVax-WN02 than YF 17D virus. There were no abnormalities in hematology and clinical chemistry, and no histological changes were observed in any examined peripheral organ of cynomolgus monkeys following both IC and SC inoculation. Post-inoculation viremia was lower compared with YF 17D in rhesus monkeys, but higher in cynomolgus monkeys, yet within the WHO specifications established for YF 17D vaccine (110,111). The latter observation was associated with a more pronounced early replication of ChimeriVax-WN02 in the skin inoculation site and lymph nodes. Generally, the biodistribution in monkeys of both ChimeriVax-WN02 and YF 17D viruses was similar, as demonstrated using sensitive quantitative PCR. Prominent sites of replication were skin and lymph tissues (as well as the spleen for YF 17D), generally sparing vital organs including the brain (111). The chimera was highly immunogenic and protected immunized monkeys from lethal IC challenge with a high dose (5 $\log_{10}$ pfu) of WN NY99 virus (110). Immunized hamsters were also protected (112).

In the first phase I clinical trial in healthy adults, the incidence of adverse events in subjects receiving the ChimeriVax-WN02 vaccine (5 $\log_{10}$ pfu, $n = 30$; and 3 $\log_{10}$ pfu, $n = 15$) was similar to the placebo group. Transient viremia was detected in most subjects. All vaccinees developed neutralizing antibodies to WN, and the majority developed WN-specific T-cell responses. Neutralizing antibody response peaked on day 21 at mean titer of approximately 6000 in the 5 $\log_{10}$ dose group, then dropped to approximately 1280 by day 28, and remained stable at this very high level until day 365, which was the last day of the study (111). Phase II safety/immunogenicity studies, including in the elderly representing the main target population, are currently underway.

Either *Culex* and *Aedes* mosquitoes, including species transmitting WN in the United States, could not be infected by the chimera, or the virus failed to spread to head tissue (113). The virus also failed to infect chickens and fish crows (114). Thus the chimera is highly unlikely to enter a natural transmission cycle with mosquito vectors and birds as amplifying hosts.

CONCLUSION

Recent advances of molecular biology have opened doors to the development of new recombinant live flavivirus vaccines. These are currently championed in terms of their high safety and efficacy demonstrated in both animal models and humans by the ChimeriVax vaccine candidates that are based on the most effective and safe flavivirus backbone, that of YF 17D vaccine virus. Within the next few years, it is anticipated that some of the described vaccines, for example, the more clinically advanced ChimeriVax-JE, will be licensed products in use as public health tools preventing human disease and saving lives.

REFERENCES

1. Lindenbach BD, Thiel H-J, Rice CM. Flaviviridae: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, et al., eds. Fields Virology. 5th ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer, 2007:1101–1152.
2. Burke DS, Monath TP. Flaviviruses. In: Knipe DM, Howley PM, Griffin DE, et al., eds. Fields Virology. 4th ed. Philadelphia: Lippincott Williams and Wilkins, 2001:1043–1126.
3. Gubler DJ, Kuno G, Markoff L. Flaviviruses. In: Knipe DM, Howley PM, Griffin DE, et al. eds. Fields Virology. 5th ed. Philadelphia: Lippincott Williams and Wilkins, Wolters Kluwer, 2007:1153–1252.