virus, it was not neuroinvasive when inoculated into mice by the peripheral route (62,67). The virus was immunogenic in mice. Because it remained neurovirulent for mice after IC inoculation, another chimera was constructed that contained the prM-E genes from LGT virus (68). LGT virus is less virulent for humans than other members of the TBE serocomplex, and immunity to LGT is cross-protective against TBE. In contrast to the DEN4/TBE virus, DEN4/LGT replicated well only in mosquito cells and had to be adapted to mammalian cells by multiple passages. Attempts to generate other chimeric variants containing the LGT-specific C-prM-E, NS1-NS2A, NS1-NS2A-NS2B-part of NS3, and NS2B-NS3 cassettes failed, again indicating that the prM-E genes are the only easily interchangeable genes. Mouse neurovirulence of this chimera was significantly reduced compared with the parental LGT virus and DEN4/TBE (68,69). Immunization of mice (69) and monkeys (70) with DEN4/LGT protected the animals from subsequent challenge with highly virulent TBE virus. The chimera did not replicate in non-hematophagous mosquitoes *Toxorhynchites splendens*, which are highly permissive for DEN viruses (70).

The DEN4 backbone has also been used to create a DEN4/WN chimeric vaccine candidate against WN. The chimera was found to be highly attenuated and immunogenic in mice and rhesus monkeys, particularly its variant with the Δ30 deletion in the 3′UTR described above (71,72), and the DEN4Δ30/WN virus is about to enter human clinical trials (73). This candidate failed to infect geese, suggesting that chimerization of WN with DEN4 resulted in complete attenuation for avian hosts (73). Its replication in several species of mosquitoes, including *Culex tarsalis* mosquitoes that are able to transmit WN virus, was generally restricted. However, the chimera was as infectious as wild-type WN for *A. albopictus* mosquitoes, a species that was introduced into the United States in the 1980s (54).

Another chimeric WN vaccine candidate was constructed using the DEN2 PDK-53 backbone. This chimera was shown to be attenuated and immunogenic in the murine model. It efficiently protected immunized mice from a high-dose WN virus challenge (74). One important safety aspect with DEN-based vaccine candidates will be to ascertain that cellular immune responses against backbone proteins do not prime some vaccinees for DHF/DSS, the severe DEN illness, if they are infected with a DEN virus type different from the type of chimeric vaccine backbone. This will be important for individuals traveling to DEN endemic countries who had been vaccinated, for example, with DEN4/TBE against TBE in Europe or Russia, or with DEN2/WN against WN in the United States.

CHIMERIVAX VACCINES

Central to the ChimeriVax® technology is the use of the best flavivirus backbone available, that of YF 17D vaccine virus. This backbone is the main prerequisite for safety and efficacy in humans and low-level replication in mosquitoes precluding uncontrolled dissemination in nature, while heterologous envelopes provide robust humoral and cellular immunity against target pathogens. The ChimeriVax vaccines against JE (ChimeriVax-JE), DEN (ChimeriVax-DEN), and WN (ChimeriVax-WN) were developed by Sanofi Pasteur (formerly Acambis Inc., Cambridge, Massachusetts, U.S.) in collaboration with many colleagues from industry, academia and the U.S. government, as well as clinicians, worldwide. ChimeriVax-JE was developed in collaboration with St. Louis University (SLU, St. Louis, Missouri, U.S.) and Baxter (Deerfield, Illinois, U.S.). ChimeriVax-DEN was developed in collaboration with SLU. Early preclinical studies on ChimeriVax-DEN and ChimeriVax-WN were supported by NIH grants. These vaccine candidates were highly effective in animals and humans. Currently, they are in phases II and III clinical trials, and thus are the most advanced in terms of testing in humans among all chimeric vaccines under development. ChimeriVax viruses infect dendritic cells, as shown for ChimeriVax-DEN chimeras, which is a prerequisite of a robust, long-lasting immunity (75, 76). In fact, ChimeriVax-DEN viruses infect dendritic cells more efficiently than YF 17D, and stimulate their maturation. This results in the induction of immunostimulatory cytokines, which is consistent with clinical observations of safety and immunogenicity (76). Among DEN vaccines under development, the tetravalent ChimeriVax-DEN1-4 vaccine was the first for which protective efficacy was demonstrated in a monkey challenge model against all four DEN types (77), and among WN vaccines, ChimeriVax-WN was the first to enter human clinical trials, with promising results. The expected product profiles include single-dose application, low rates of (mild) adverse events, and rapid-onset, durable immunity that fit the current needs for vaccines for both travelers and main target populations in endemic countries (e.g., children or the elderly). In addition, the ChimeriVax technology has been applied by Intervet Inc. to develop a live, attenuated chimera vaccine against WN virus for use in horses. The single-dose equine vaccine has been shown to protect horses against severe intrathecal challenge with wild-type WN virus (78–80), and is now commercially available. A ChimeriVax-SLE chimera has been genetically engineered and, if necessary, could be further developed as a vaccine against SLE virus that causes sporadic disease outbreaks in South and Central America and southern and central U.S. states (81). It has been distributed along with ChimeriVax-WN chimera by the CDC to State Health Department Laboratories for diagnosis and epidemiological surveillance of WN and SLE.

The biological properties of ChimeriVax vaccines in animal models as well as humans are exemplified by those for ChimeriVax-JE in Table 2. The characteristics are generally representative of other ChimeriVax candidate vaccines. It should be noted that new serious adverse events associated with YF 17D vaccination have recently come to light with improved surveillance, such as adverse neurotropic disease in adults (incidence ~1.3–2.5/1 million) and adverse viscerotropic disease resembling classical YF (incidence ~2.5/1 million, which could be higher in the elderly) (8). Although rare, these adverse reactions indicate that an improvement in the YF 17D vaccine safety may be necessary, for example, using molecular manipulations. Importantly, the results described below show that ChimeriVax vaccines are more attenuated compared with YF 17D. For instance, neurovirulence of ChimeriVax viruses in mice and monkeys is significantly lower compared with YF 17D, and it is unlikely that chimeras will be able to cause YF-like symptoms.

ChimeriVax-JE

Early reports on the construction of intertypic DEN chimeras and DEN4/TBE were a prelude to the ChimeriVax technology illustrated in Figure 2. It started with the work of Chambers and coworkers, who succeeded in the construction of first YF 17D chimeras containing the prM-E genes (but not C-prM-E)