from a wild-type JE virus (Nakayama) or the SA14-14-2 vaccine strain (82). The YF 17D/SA14-14-2 chimera was avirulent in young mice by both IC and intraperitoneal (IP) routes at all tested doses up to 6 $\log_{10}$ pfu, and significantly less neurovirulent in suckling mice compared with YF 17D virus that is lethal for mice of all ages inoculated by the IC route (Table 2). The chimera induced solid immunological protection against challenge with a highly virulent strain of JE virus (83). Importantly, protection in mice was achieved against wild-type viruses belonging to all four major JE virus genotypes (84). The YF 17D/SA14-14-2 chimera was selected as a primary vaccine candidate designated ChimeriVax-JE. This virus grew to high titers, in excess of 7 $\log_{10}$ pfu/mL, in Vero cells acceptable as a substrate for human vaccine manufacture. It also replicated efficiently in tested simian, human, mouse, and mosquito cells. The virus had the antigenic specificity of JE virus, that is, was neutralized by JE-specific antibodies but not by YF-specific antibodies. It was found to be highly stable genetically and phenotypically. Mouse neurovirulence of the virus did not increase following 18 passages in cell culture (MOI 0.1 pfu/cell) or six mouse brain-to-brain passages (83). In a separate study, it was demonstrated that multiple simultaneous reversions to the JE Nakayama sequence in distinct clusters of the E protein were required for reversion of ChimeriVax-JE to a higher neurovirulence in mice (85). Yet, in contrast to JE Nakayama virus, YF 17D/Nakayama chimera was not neuroinvasive, and its mouse neurovirulence profile was similar to YF 17D (82).

Extensive testing of ChimeriVax-JE in rhesus monkeys demonstrated that the vaccine virus was highly attenuated for this primate species and less pathogenic than the YF 17D vaccine in all standard tests (86,87). The virus induced a low, self-limited viremia following both IC and SC inoculation, similar to viremias induced by 17D. This is an important feature that minimizes the possibility of neuroinvasion and encephalitis in vaccinees, and reduces the chances of virus spread in nature by feeding mosquitoes. Immunization with doses of ChimeriVax-JE vaccine, as low as 2 $\log_{10}$ pfu and up to 5.3 $\log_{10}$ pfu, elicited high titers of JE-specific neutralizing antibodies of 1:640 to 1:1600 after a single SC inoculation. Animals that received the vaccine were protected from a severe IC challenge with a highly virulent wild-type JE virus strain (86,87) (Table 2). In addition to the standard SC inoculation, the vaccine can be delivered to the epidermis, by skin microabrasion, eliminating the need for needles (88).

ChimeriVax-JE has been shown to be well tolerated and highly immunogenic for humans vaccinated with the virus in three phases I and II clinical trials. In one trial, administration of a single dose of 4 or 5 $\log_{10}$ pfu of the virus to YF-immune and naïve volunteers caused no serious adverse events (89). The rates of mild, transient injection site reactions and flu-like symptoms were similar to control groups of subjects that received the YF 17D vaccine. Subjects inoculated with the chimera in both dose groups developed a transient, low-titer viremia similar in magnitude and duration to that following 17D immunization. The rates of seroconversion to JE were 100% in both high- and low-dose groups in both naïve and YF-immune subjects. The mean JE-specific neutralizing antibody titers were higher in the high-dose groups (1:254 and 1:327 in naïve and YF-immune subjects, respectively) than in the low-dose groups (1:128 and 1:270 in naïve and YF-immune subjects, respectively), and also higher in YF-immune than naïve individuals (Table 2). These data dispel the concern that YF 17D anti-vector immunity could limit the usefulness of ChimeriVax vaccines in regions where the general population were either immunized against YF or were infection immune. Anti-vector immunity in the case of ChimeriVax viruses could involve cytotoxic T-lymphocyte responses to YF 17D virus NS proteins or cytolytic antibodies against NS1. For comparison, vaccinia recombinants expressing JE virus immunogens failed to induce JE neutralizing responses in vaccinia-immunized subjects (90).

In another clinical trial, 10 subjects vaccinated with ChimeriVax-JE were challenged with one standard dose of formalin-inactivated JE vaccine (JE-VAX) as a surrogate for exposure to live virus. The vaccinees demonstrated a significant rise in JE virus-specific neutralizing antibodies (100-fold on day 14 post-challenge), while the control naïve participants showed no or barely detectable antibody levels. Thus, a strong anamnestic immune response, an important prerequisite of vaccine effectiveness, was observed in the vaccinated individuals.

In a third study, the ChimeriVax-JE vaccine was equally effective in subjects immunized with five different graded doses ranging from 1.8 to 5.8 $\log_{10}$ pfu ($n = 11$ to 44 per group), and sera from vaccinees efficiently neutralized Japanese, Chinese, and Vietnamese wild-type strains of JE virus (91). Collectively, these data from clinical trials demonstrate an excellent safety and efficacy profile for ChimeriVax-JE in humans. Phase III safety and efficacy clinical trials have been completed showing an acceptable safety profile and non-inferior immune response of one dose of ChimeriVax-JE to three doses of inactivated mouse brain JE vaccine, and a pediatric study in children in India is underway. A license application for this vaccine is in preparation.

An important feature of a successful vaccine is that it is safe for the environment. Similar to the YF 17D virus, and in contrast to the SA14-14-2 parent vaccine virus, ChimeriVax-JE has been shown to be unable to infect *Aedes* and *Culex* mosquitoes by oral feeding (92,93). This observation, together with low, short-lived post-inoculation viremia in humans and animals, which is insufficient for infecting feeding mosquitoes, virtually eliminates the possibility of uncontrolled dissemination of the vaccine virus in nature. In contrast, oral polio vaccine viruses, for example, readily spread and recombine with natural polioviruses. In terms of the theoretical possibility of recombination of ChimeriVax vaccines with endemic flaviviruses, it is further minimized by the fact that there are no known, confirmed examples of recombination between flaviviruses in nature, even among the genetically close DEN virus types (94,95). The YF 17D vaccine has been widely in use for 70 years, and there has been no evidence of its uncontrolled spread or recombination with any wild-type flavivirus, including YF. To demonstrate experimentally that recombination in nature between a wild-type flavivirus and a ChimeriVax vaccine virus would result in a recombinant with little potential to cause disease or even survive in nature, artificial recombinants between ChimeriVax-JE and an Australian virus KUN were constructed. The resulting chimeras proved highly attenuated in comparison with the KUN parent. They replicated very poorly in mice and hamsters, were not neuroinvasive, and their neurovirulence in mice was similar to YF 17D and significantly lower than KUN (96). These results further strengthened the point that any recombinants, should they ever emerge, would have little chance to cause disease or spread by successfully competing in nature with wild-type flaviviruses.