A critical appraisal of Ixiaro® – a cell-derived inactivated vaccine for Japanese encephalitis

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Abstract: Japanese encephalitis is a disease prevalent across a huge swathe of southeast Asia. The number of reported cases of the disease is increasing in countries that do not have a vaccination program, but in contrast, is decreasing in countries that have implemented mass vaccination programs. Clearly vaccination is having some impact, and although visitors to the area are generally thought to be at low risk, vaccination is recommended for those staying 1 month or longer. Until recently, the only licensed vaccine available to them, JE-VAX®, was made from virus propagated in mouse brain, and among Western Hemisphere recipients of this vaccine, many side effects and adverse events were reported, and production of the vaccine was discontinued in 2007. A new vaccine, Ixiaro®, has recently been licensed. The vaccine comprises inactivated virus, previously propagated in Vero cells, adsorbed onto an alum adjuvant. In extensive clinical trials in both adult and pediatric populations, Ixiaro® has proven non-inferior to JE-VAX® in terms of immunogenicity and seroconversion, but with an improved safety and tolerability profile compared with JE-VAX®.

Keywords: vero cells, JEV, vaccine

Prevalence and spread of Japanese encephalitis virus

There are over 500 hemophagic arboviruses (arthropod-borne viruses) of which some 150 cause human disease.¹ Although most arbovirus infections do not develop into full blown disease, when it occurs, symptomatic disease usually presents with 1 of 3 different syndromes: febrile illness, hemorrhagic fever, or neuroinvasive disease (eg, encephalitis, meningitis or myelitis).¹ Flaviviridae are one of the taxonomic groups of arboviruses transmitted mostly by ticks or mosquitoes;² therefore they are found in most regions of the world.³ Viruses in this group cause diseases such as Japanese encephalitis (JE), Murray Valley encephalitis, St. Louis encephalitis, tick-borne encephalitis, West Nile encephalitis, dengue fever and yellow fever.^{2,3} As with all arboviruses, flaviviruses are maintained in zoonotic cycles involving birds and small mammals; with the exceptions of dengue and yellow fever viruses, humans and domestic animals are incidental, ie, dead end, hosts.^{2,3}

Japanese encephalitis virus (JEV) is a typical flavivirus. It replicates in a zoonotic cycle involving mosquitoes (principally *Culex tritaeniorhynchus*, although depending on the specific area, other culicine species may be involved), wading birds which are the natural reservoir for the virus, and pigs which serve as amplification hosts.⁴ In the particular case of JEV, horses as well as humans are incidental hosts – there

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have been no demonstrable instances of transmission from person to person.⁴ JEV is also a typical flavivirus in the spectrum of disease it causes; viral infection results in a range of clinical manifestations from asymptomatic infection with no disease, through mild febrile illness, to acute and lethal encephalitis.⁵ Fortunately the incidence of severe neuroinvasive disease is low with estimates of between 1 in 50 and 1 in 1000 infections resulting in encephalitis.⁵ Nonetheless JEV is the leading cause of viral encephalitis in Asia with an estimated 45,000 cases per annum, mainly in the young, elderly or immunocompromised.⁵ However there are reports that these numbers may be significantly higher – in the areas where JEV is prevalent, disease surveillance is suboptimal, leading to suggestions that the true annual incidence is closer to 175,000.^{4,6,7} Case fatality rates are typically between 20% and 40%, meaning there are at least 10,000 deaths annually, but on occasions the rate can exceed 60%.^{4,6} Infection with JEV confers life-long immunity, and by the time they reach adulthood, most people living in the endemic areas have been exposed to JEV and have seroconverted to the virus.⁸

The mosquitoes that carry JEV are found in tropical and temperate areas of Asia, in a swathe of countries bounded by India in the west and Japan in the east, southern Siberia in the north and the northernmost tip of Australia.⁴ It has been estimated that almost half the world's population inhabits this area and therefore a huge number of people are potentially at risk from infection.^{7,9} In the last couple of decades, this area has also seen a massive increase in tourism, and while most tourists tend to stay in cities and are therefore at little risk from infection, there are still substantial numbers of people who venture out of cities and who could therefore become infected. In the tropical areas JEV infection is endemic while in the subtropical and more temperate climates, infection is epidemic with peak incidence occurring during the rainy season when proliferation of mosquitoes is at its highest.⁴ With the increased demands for food to feed an ever expanding population, pig husbandry has increased at an enormous rate, as too has irrigation of land for rice paddies (increasing overall in the area by 50% and 8% respectively between 1990 and 2005).⁹ It is no surprise therefore that infections are most common in rural areas where extensive rice paddies in close proximity to pig farms provide near perfect conditions for mosquitoes to breed.⁴

Management issues in the prevention of Japanese encephalitis

Attempts to control JEV infection and limit the spread of the virus have naturally sought to reduce the population of

mosquitoes or limit human exposure to them by appropriate dress, use of insect repellents and installation of anti-insect screens in dwellings.^{3,4,9} However vector control is expensive and requires an infrastructure that simply does not exist in many of the affected areas. Chemicals in the form of pesticides, insecticides and/or larvicides are also expensive and tend to play a marginal role in the routine control of JEV carrying mosquitoes and are frequently reserved for emergency use only.⁴ Furthermore, resistance to these chemicals is increasing which can only compromise their use in emergency situations.⁴

Environmental management has also been suggested as a means of mosquito control. Alternative irrigation methods (alternating wet and dry irrigation) have had some limited success, as has the introduction of larvivorous fish.⁴ Physical separation of humans, pigs and rice paddies is another way of controlling JEV infections, and where this separation occurs naturally such as in Malaysia, the absolute numbers of reported infections are certainly significantly lower than in other countries where it is the norm for pigs and paddy fields to be found admixed with human populations; there may however be other reasons for this – vaccination rates for example might be higher.^{3,9} Vaccination of piglets has also been suggested though this is an expensive and largely futile exercise since piglets cannot be vaccinated until they are 6 months old – the only vaccine available is a live attenuated virus and the maternal antibodies piglets possess until they are about 6 months old would render the vaccine ineffective – and they are usually slaughtered 2 months later.^{3,4}

Epidemiological data show 2 trends in the incidence of JEV infection. In countries where there is little or no disease surveillance or monitoring, and few or limited vaccination programs, the incidence of disease is increasing. In contrast, in countries such as China, Japan, South Korea and Thailand which are relatively affluent and have active surveillance and vaccination programs, the incidence of JE is either stable or falling.⁹ This information suggests that while it is just one factor of many in a complex interplay of different social, economic, environmental and clinical elements (not to mention the largely unpredictable effects of climate change), vaccination of at-risk populations and especially children, must play a significant role in the control of JEV infections.

The virus and the disease

JEV is a small (positive sense) single stranded RNA virus of the genus *Flavivirus*.¹⁰ It has a lipoprotein envelope surrounding a nucleocapsid and core; the genome is packaged in the capsid formed by the capsid protein (C). The viral outer

membrane contains an envelope protein (E) which is believed to facilitate entry of the virus into its host cell and which has been shown to be the protective antigen. The genome also encodes a membrane protein (M), and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) of which NS3 is thought to be a helicase while NS5 is thought to be a polymerase. There are 5 genotypes of the virus and only a single serotype.

In animal studies, it has been shown that neural progenitor cells (NPC) are permissive to JEV infection both *in vitro* and *in vivo*, causing abnormalities in their growth and a decrease in the number of actively proliferating NPCs in the subventricular zone of infected animals.¹¹ It has been suggested that if this reflects what occurs in humans, it may be a primary cause of the dysregulated neurogenesis observed in people suffering from JE. Similarly, other animal studies have demonstrated recruitment or depletion of microglia in different areas of the brain with concomitant differential expression of proinflammatory cytokines and chemokines in these different areas.¹² It has been proposed that the dysregulated production of cytokines and chemokines may be deleterious to the post-mitotic neurons in brain tissue by causing an autotoxic loop of microglial activation which may in turn lead to bystander neuronal damage.

JE is diagnosed by detection of specific IgM antibodies in serum or CSF to differentiate JEV from other causes of encephalitis such as herpesviruses, other flaviviruses, or other pathogens which cause neuroinvasive disease such as bacterial meningitis, tuberculosis or cerebral malaria.⁶ Infection with JEV is most frequently subclinical with few infections (estimates range from only 1 in 50 to 1 in 1000) developing into encephalitis.⁸ Symptomatic disease has an incubation period of 5 to 15 days and initially presents as a non-specific systemic febrile illness which may include headache, cough, nausea and vomiting, and which could have multiple etiologies.⁸ Neurologic symptoms quickly follow marked by coma, seizures, Parkinson-like movement disorders, meningitis or flaccid paralysis. The case fatality rate is between 20% and 40% overall but is generally much higher in children. Of the survivors, between 25% and 50% suffer from long term or permanent neurologic or psychiatric sequelae with physical or mental impairments.⁶ These may include severe cognitive and/or language impairment, deafness, emotional instability, motor deficit or hemiparesis. Only about a third of survivors show no sequelae at all.⁶ There is no specific treatment for Japanese encephalitis and treatment is palliative and supportive, depending on the symptomology.⁶ Preventing infection, principally through vaccination, is

therefore the most important and effective countermeasure against JEV.

Vaccines against JEV infection

Until recently, there were three vaccines available against JEV infection, all based on the genotype 3 strains of virus. One is a cell-culture derived inactivated whole virus vaccine; a second is a cell-culture derived live attenuated vaccine; and a third is a mouse-brain derived inactivated whole virus vaccine.¹⁰ Only the last was available internationally; the other two vaccines were available only in China and neighboring countries.

The cell-culture derived inactivated whole virus vaccine was originally made by propagating the Beijing P-3 strain of virus in primary hamster kidney (PHK) cells.¹³ The virus was isolated and then inactivated by treatment with formalin. It was widely used throughout China and was highly immunogenic although its use was associated with a low frequency of transient local reactions. Mild systemic reactions were also reported, likely due to the gelatin the vaccine contained (see below). In attempts to standardize the manufacturing process and conform to World Health Organization (WHO) guidelines, the PHK cells have in recent years been replaced by Vero cells.

The cell-culture derived live attenuated virus vaccine has been produced in China for 20 years.^{10,13} The virus is also propagated in PHK cells but in order to gain wider licensure of this vaccine, the manufacturer is complying with the WHO's production standards and Vero cells are increasingly being used to manufacture this vaccine. Furthermore there is enhanced screening to ensure the vaccine is free from adventitious agents. The vaccine is based on the SA₁₄-14-2 strain of virus, and has recently been made available in Nepal, India, Sri Lanka and South Korea. The vaccine has proven to be highly immunogenic, eliciting broad protective immunity against heterologous JEV, and has been shown to be safe and effective in children with an efficacy of nearly 100% after 2 doses administered at 1 and 2 years of age.

The mouse-brain derived inactivated virus vaccine was originally made in Japan in the 1930s and was available internationally – Sanofi-Pasteur distributed the vaccine in the US and Europe as JE-VAX®.^{10,13} The vaccine used either the wild-type Nakayama (for the internationally available vaccine) or Beijing-1 strains of virus, and was made by purifying virus from the brains of intracerebrally inoculated mice. After purification, the virus was inactivated by treatment with formalin and bovine or porcine gelatin was added as a stabilizer. The vaccine was given as 2 doses

Table I The clinical trials in which the safety and immunogenicity of Ixiaro® have been tested. All the trials were conducted in adult populations unless specifically mentioned

Trial	Phase	Endpoints	Outcomes	Ref
WRAIR 815	II	Dose finding; safety and tolerability using JE-VAX® as comparator	Dose and regimen were established ($2 \times 6 \mu\text{g}$ im on d0 and d28); Ixiaro® was safe, well tolerated and at least as immunogenic as comparator	40
	II	Safety and immunogenicity in 1- to 3-year-old infants using JE-VAX® as comparator	$3 \mu\text{g}$ of Ixiaro® was safe and highly immunogenic in children, and prevented childhood encephalitis	48
IC51-301	III	Safety; non-inferiority vs JE-VAX®	Ixiaro® was non inferior to JE-VAX®; pre-existing anti-TBEV immunity did not impair induction of anti-JEV immunity	41,44
IC51-302	III	Safety in recipients of Ixiaro® or placebo	Incidence of mild to moderate adverse events were similar in recipients of Ixiaro® and placebo	42
IC51-303	III	Safety and immunogenicity follow up over 24 months in volunteers from IC51-301 and IC51-302	Frequency of adverse events was similar in recipients of Ixiaro®, JE-VAX® and placebo; immunogenicity of Ixiaro® was durable over 24 months	43
IC51-304	III	Immunogenicity with a rapid immunization regimen ($1 \times 12 \mu\text{g}$ Ixiaro®)	1 dose of $12 \mu\text{g}$ Ixiaro® was inferior to 2 doses of $6 \mu\text{g}$	45
IC51-305	III	Persistence of immunity in seroconverters from IC51-304 or in boosted non-seroconverters	Results not yet available	
IC51-308	III	Non-inferiority of Ixiaro® co-administered with Hep A vaccine	No interference in induction of anti-hep A or anti-JEV antibodies	46,47
IC51-309	III	Batch consistency	Similar immunogenicity indicated consistency of batches	
IC51-310	III	Consistency of commercial batches	Similar immunogenicity indicated consistency of batches	
IC51-311	III	Long-term follow up of IC51-309 volunteers and effects of boosting	Results not yet available	
IC51-314	III	Long-term assessment of immunogenicity post filing	Results not yet available	

to 1- to 3-year-old children in endemic regions, and as 3 doses to travelers to the regions. The vaccine was highly immunogenic, eliciting durable protection in the former population for up to three years post immunization, but disappointingly, less durable protection (<1 year for 70% of vaccinees) in the latter.^{10,13,14} However, there were safety concerns over the possibility of neuronal disease caused by contaminating mouse brain proteins, as well as the addition of gelatin, which has been associated with allergic reactions in susceptible vaccinees.^{7,8,10} Although no specific vaccine constituent has been identified, it is suspected that gelatin may be responsible for the mild to moderate local and systemic side effects – fever, headache, malaise, rash, myalgia, nausea – experienced by up to 20% of vaccinees.^{14–19} Patterns of adverse reactions including urticaria, facial or

oropharyngeal angioedema have also been documented following use of this vaccine, particularly in European and North American travelers to endemic regions,^{13,14} and rarely, fatal cases of acute disseminated encephalomyelitis (ADEM) have been reported in children in endemic areas as well as in travelers to the region.^{20–23} Following a case of ADEM in Japan in 2005 which may or may not have been related to administration of the vaccine, routine JEV vaccination was suspended and production of this vaccine ceased.²³

Reduction of the incidence of JE in China, Japan, South Korea and Thailand following mass vaccination campaigns, has demonstrated the efficacy of these programs in their at risk populations. While there was thought to be little risk to short-term tourists to these areas and that therefore vaccination may not be needed, there have been documented instances of

infections in tourists to endemic areas with estimates of 1 to 2 JEV infections per million travelers.^{24–28} Bearing in mind the considerable number of reports of side effects, allergic reactions, anaphylaxis and even severe adverse events that were associated with the use of JE-VAX®,^{13–23} Health Authorities erred on the side of caution and provided a restricted recommendation that travelers intending to stay in endemic regions for a period of a month or longer should consider themselves at greater risk and should therefore consider receiving the only available vaccine, JE-VAX®, despite the rare potential for adverse reactions.²⁹

Since production of JE-VAX® ceased the world's stockpiles of JE vaccine have become severely depleted, signaling a major concern to the WHO which has consequently placed a high priority on the development of new vaccines for the prevention of JE. Two new JE vaccines, ChimeriVax™-JE and Ixiaro® (previously known as IC-51) have been in development for a number of years and Ixiaro® has recently been licensed in the US, Australia and in Europe. A third vaccine, MVA-BN-JE, is currently in preclinical development.¹⁰

Ixiaro® is a product of Intercell AG based in Vienna. In April, 2003, Intercell AG entered into agreements with VaccGen International LLC to acquire a Japanese encephalitis virus vaccine project which would allow it to further develop the vaccine and to market it after successful completion of the development process and regulatory approval. In November 2004, Intercell AG obtained a worldwide non-exclusive license from Aventis Pasteur S.A. (now Sanofi Pasteur S.A.) for certain I.P. related to the Japanese encephalitis virus vaccine. The US patent, US-06309650,³⁰ was granted to CJ Corp and the Walter Reed Army Institute of Research (US Army) and claims attenuated JEV adapted to growth in Vero cells by passage in Vero tissue culture. It expires in August 2018. It is predated by an International Patent Application, WO/99/11762,³¹ and European patent application EP-01604685,³² which claim vaccines containing JEV and an aluminum hydroxide adjuvant.

Intercell AG has established strategic alliances with Novartis, Sanofi Pasteur as well as Merck to market and distribute its vaccines. Intercell AG acquired Iomai in 2008, and with it, the technology platform for transdermal delivery of vaccines by means of patches.

Manufacture of Ixiaro®

Two principal issues concerning the only available vaccines against JE were: 1) the propagation of virus in either a relatively uncharacterized primary cell substrate (PHKs) or a relatively uncontrolled and uncharacterized live animal tissue

(mouse brain), and 2) the use of gelatin as a stabilizer with consequent issues of lot-to-lot heterogeneity and allergenicity in some vaccinees.

To overcome the first issue, Intercell AG acquired the rights from VaccGen International LLC, to an attenuated SA₁₄-14-2 strain of JE which had been adapted to grow in Vero cells. Vero cells have become widely accepted as a substrate for the production of vaccine viruses.³⁰ Regulatory concerns require that any cell considered for production of human viral vaccines must have a documented source and history, must be free of retroviruses, mycoplasma, bacteria, fungi, mycobacteria or any other adventitious agent, and must be tested for tumorigenicity and oncogenicity.³³ GMP quality Vero cells have been extremely well characterized in recent years and have a relatively long history of use in the production of human viral vaccines such as the polio and rabies vaccines.³⁴ It was a logical step therefore to expect that Vero cells might be a suitable cell substrate for propagation of JEV for vaccine purposes.

The virus was obtained after passaging the SA₁₄ wild-type JEV in mice followed by passage in PHKs, primary dog kidney cells and then in Vero cells.^{30,35,36} Following infection of Vero cells, cells were incubated for 2 or 3 days and virus was harvested from the culture supernatants by low speed centrifugation and filtration (by harvesting the virus before cytopathic effects were observed, contamination of the virus by host cell proteins was minimized). The virus preparation was then concentrated by tangential flow ultrafiltration of the supernatant/filtrate, addition of protamine sulfate followed by high speed centrifugation ensured removal of residual DNA, and virus was finally purified by centrifugation on sucrose gradients. Purified virus was then formalin-inactivated and alum adjuvant was added. Manufacture of the vaccine was performed under good manufacturing process (GMP) using a well characterized cell substrate, and since the vaccine was inactivated and adsorbed onto alum adjuvant, there was no requirement to add gelatin, thereby sidestepping the second issue. Addition of alum adjuvant is unlikely to introduce any new issues as alum has over 80 years of demonstrable safe use in human and veterinary vaccines.^{37,38} In addition clinical trials conducted so far have shown that Ixiaro® has a good safety and tolerability profile (see below).

Preclinical studies with Ixiaro®

The immunogenicity of Ixiaro® was tested in a dose escalation study in groups of 10, 4- to 5-week-old outbred female mice given 1 ng, 6 ng, 32 ng, 160 ng or 800 ng of

vaccine subcutaneously. Control mice received 2 ng, 10 ng, 48 ng, 240 ng or 1200 ng of JE-VAX[®] subcutaneously.³⁶ All mice received 2 doses of either vaccine on days 0 and 28 and were bled on day 42. The immunogenicity of the vaccines was assessed by determining serum anti-JEV neutralizing titers, reported as an immunizing dose 50% (ID₅₀), this being the dose of vaccine needed to elicit detectable titers of neutralizing antibody (a 50% plaque-reduction neutralization test or PRNT₅₀ > 1:10) in 50% of immunized mice (Figure 1A). For Ixiaro[®], the ID₅₀ was 4 ng (95% confidence interval [CI] 2–7 ng) compared with 15 ng (95% CI 14–17 ng) for JE-VAX[®]. All mice inoculated with ≥32 ng of Ixiaro[®] seroconverted with neutralizing titers above the level considered protective in humans (PRNT₅₀ > 1:10). Only 70% of mice given JE-VAX[®] had seroconverted at the 48 ng

dose, although at the next incremental dose tested (240 ng), all mice seroconverted.³⁶

The protective efficacy of Ixiaro[®] was tested³⁶ in groups of 10, 3-week-old mice immunized subcutaneously with 1.5 ng, 15 ng, 150 ng or 1500 ng or 0.6 ng, 6 ng, 60 ng, 600 ng of JE-VAX[®]. Mice were immunized with either vaccine on days 0 and 14 and were then challenged by intraperitoneal injection of approximately 1000 median lethal doses (1000 LD₅₀) of the parent virus (SA₁₄) on day 21. At the time of challenge, mice were also injected intracranially with 30 µL of saline to disrupt the blood–brain barrier and all mice were monitored for death or signs of encephalitis for 14 days post challenge. Immunization of mice with 15 ng Ixiaro[®] elicited an 80% survival rate, whereas immunization with ≥150 ng elicited 100% survival (Figure 1B). Similar data were obtained

Figure 1 The plaque reduction neutralizing titers (PRNT₅₀) induced by immunization of mice with different doses of Ixiaro[®] or JE-VAX[®]. **A**) the log of the geometric mean titers (GMT) (and range of titers) induced by different doses of either vaccine; **B**) the log of the geometric mean titers induced by different doses of either vaccine (bars) and the number of mice (%) which survived challenge with live JEV (lines).

by immunization with JE-VAX® when administration of 6 ng of vaccine elicited 90% survival and administration of ≥ 60 ng offered complete protection. The effective dose of vaccine (ED_{50} – the dose required to protect 50% of immunized mice) was calculated to be 2.6 ng (95% CI 0.2 ng–7.6 ng) for Ixiaro® and 1.5 ng (95% CI 0.6–3.7 ng) for JE-VAX®.

Protection elicited by passive transfer of serum was also assessed.³⁹ Mice were injected intraperitoneally with either 0.5 mL of high- ($PRNT_{50} = 1:256$) or low-titered ($PRNT_{50} = 1:26$) serum pooled from human volunteers vaccinated with Ixiaro® using a standard regimen, or non-immune serum. All mice were challenged 18 hours later with 50 LD_{50} SA₁₄ after disruption of the blood brain barrier by intracranial saline injection. At the end of the 21-day monitoring period, none of the mice in the high $PRNT_{50}$ group and only 1 of 10 mice in the low $PRNT_{50}$ group developed any sign of disease whereas 9 of 10 mice given non-immune serum had either died or developed clinical disease. Thus, JEV neutralizing antibodies were capable of protecting mice against lethal JEV challenge.³⁹

Clinical trials of Ixiaro® – safety, immunogenicity and efficacy studies

Safety and immunogenicity phase I/II trials

A randomized, open-label, unblinded, single-center phase Ib/II dose-finding clinical trial investigated the safety, tolerability, and immunogenicity of Ixiaro®.⁴⁰ (See Table 1 for an abbreviated list of all the clinical trials of Ixiaro®.) 94 healthy volunteers were randomized to 1 of 4 groups and given a) 6 μ g Ixiaro® intramuscularly (im) on days 0 and 28, b) 6 μ g Ixiaro® on days 0, 14 and 28, c) 12 μ g Ixiaro® on days 0 and 28, or d) JE-VAX® (sc) on days 0, 7 and 28 as per the recommended schedule. By day 56, 95% of volunteers who received the lowest dose of Ixiaro® (Group a) and 100% of vaccinees in the other 2 Ixiaro® cohorts had $PRNT_{50} \geq 10$, compared with 74% of JE-VAX® recipients. One year later, 48 volunteers from the three groups given Ixiaro® and still in the trial ($n = 48$) remained seroconverted whereas only 11 (54%) of volunteers who received JE-VAX® remained seroconverted. After 2 years, all volunteers who received the highest Ixiaro® dose remained seroconverted. This trial also identified a dose and schedule – 6 μ g of Ixiaro® given on days 0 and 28 – which would become the standard regimen for administration of the vaccine in subsequent trials.

Ixiaro® has also been tested in a randomized, controlled, pediatric phase II clinical trial comparing the efficacy of

JE-VAX® and 3 μ g of Ixiaro® in 1- to 3-year-old infants in India. Preliminary data released by the company showed the vaccine was safe and immunogenic – 96% of infants given the vaccine seroconverted. By day 56, the geometric mean [antibody] titers (GMT)s in this group were of the order of 200, similar to the titers observed in phase III trials in adults (see below).

Immunogenicity phase III trials

A multicenter, observer-blinded, randomized, controlled phase III clinical trial (IC51-301) was performed to demonstrate the non-inferiority of Ixiaro® and JE-VAX®.⁴¹ 867 healthy adults were randomized to receive either two im injections of 6 μ g of Ixiaro® on days 0 and 28 (plus a placebo dose on day 7 to mask the comparator) or three doses of JE-VAX® given by the standard regimen. 98% of Ixiaro® recipients had seroconverted by day 56 with GMTs of 244 compared with 95% of JE-VAX® recipients who had seroconverted with GMTs of 102. Subject age had no significant effect on the immune responses to Ixiaro®.

The safety and tolerability of Ixiaro® was assessed in a second randomized, double-blind, placebo-controlled clinical trial (IC51-302) in 2675 healthy volunteers randomized in a 3:1 ratio to receive either Ixiaro® (6 μ g given im on days 0 and 28) or placebo.⁴² Volunteers from this as well as IC51-301, a total of 3258 subjects, were followed up for 2 years after primary vaccination (IC51-303; NCT00596102) by assessing their serum anti-JEV titers at 6 months post initial vaccination (comparing Ixiaro®, JE-VAX® and placebo), and at 12 and 24 months (non-comparative assessment).⁴³ Safety was further assessed by follow ups at 36, 48 and 60 months after the initial vaccination. At the 6-month comparative assessment stage, 95% of Ixiaro®-treated volunteers still had high seroconversion rates (SCRs), compared with 74% of vaccinees given JE-VAX®. There was a 9% SCR in volunteers given placebo. Compared with the GMTs at 56 days post vaccination, GMTs at 6 months post vaccination were reduced in both Ixiaro® and JE-VAX® groups, although the titers were twice as high (84) in those vaccinated with Ixiaro® than the titers in the JE-VAX® group (34). Only 5% of Ixiaro®-treated volunteers had a 6-month $PRNT_{50}$ below the protective titer of 10 compared with 27% with JE-VAX® recipients while at 12 months, 18% of Ixiaro® recipients were below the protective titer. 83% of the Ixiaro® vaccinees maintained high SCRs with GMTs of 41. The trial has not yet completed.

To assess whether interference from antibodies against another flavivirus affected the immunogenicity of Ixiaro®,⁴⁴ subjects from IC51-301 were sub-divided into volunteers with

or without pre-existing immunity to tick-borne encephalitis (TBE). Of the 430 volunteers treated with Ixiaro[®], 81 had anti-TBE antibodies. At day 28, after a single dose of Ixiaro[®], 77% of volunteers with TBE had seroconverted compared with 49% in those who were TBE-negative ($P < 0.0001$); similar results were seen for GMT titers. There were no significant differences in SCRs and GMTs between the groups after either 1 or 2 doses of Ixiaro[®]. In the volunteers without anti-TBE antibodies, the percentage that seroconverted was higher after 2 JE-VAX[®] vaccinations (81%) than after a single dose (49%) and SCRs were more comparable at day 56 after two doses of Ixiaro[®] (98% compared with 89% for JE-VAX[®]). These data suggested that pre-existing TBE immunity appeared to enhance the neutralizing JEV-specific antibody response. As observed previously, there were no significant differences in SCRs and GMTs following Ixiaro[®] vaccination in volunteers over 50-years-old or those younger than 50.

A clinical trial was conducted to assess whether an accelerated immunogenicity regimen – a single dose of 12 µg of Ixiaro[®] – was as immunogenic as the standard immunization regimen (IC51-304; NCT00595790).⁴⁵ 374 volunteers were randomized to one of three groups given: a) Ixiaro[®] by the standard regimen; b) 6 µg of Ixiaro[®] as a single im injection; or c) 12 µg of Ixiaro[®] as a single im injection. Only 40% of recipients of the single 6 µg dose and 66% of the single 12 µg dose seroconverted, showing that the responses were inferior to those elicited by the standard two dose regimen. IC51-305 (NCT00595270) was a follow up to IC51-304 in which the persistence of the immunogenicity of Ixiaro[®] would be assessed in volunteers who had seroconverted, while those who had not seroconverted would receive a booster dose 11 or 23 months after the initial vaccination. The trial has completed but the results have not yet been made available.

A multicenter, single-blind, randomized, controlled phase III clinical trial evaluated the immunogenic effects of concomitant vaccination of Ixiaro[®] with Havrix[®] 1440, an inactivated virus vaccine against hepatitis A, frequently given to travelers (IC51-308; NCT00596271).^{46,47} Healthy volunteers ($n = 192$) were randomized to 1 of 3 groups given: a) 2 doses of Ixiaro[®] (6 µg im, days 0 and 28) plus 1 dose of placebo (0.5 mL, day 0); b) 2 doses of placebo (0.5 mL, days 0 and 28) plus Havrix[®]1440 (1 mL im, day 0) or c) 2 doses of Ixiaro[®] (6 µg, days 0 and 28) plus Havrix[®]1440 (1 mL im, day 0). By day 56, the GMTs for anti-JEV neutralizing antibodies were comparable for vaccinees in Group c) compared with Group a) (203 compared with 192 respectively). Anti-hepatitis A titers were 24 for group c) compared with 22 for Group b). These data suggested

that there was no immune interference when Ixiaro[®] and Havrix[®]1440 were administered concomitantly.

As of September 2009, a number of other phase III clinical trials of Ixiaro[®] have been completed or were still ongoing. IC51-309 (NCT00594958) randomized 639 volunteers into one of three groups, each of which was given 1 of 3 batches of Ixiaro[®]. This trial dovetailed with IC51-310 (NCT00595465), in which 389 volunteers were randomized to 1 of 3 groups each of which was given 1 of 3 commercial batches of Ixiaro[®]. Both trials were performed to demonstrate batch to batch consistency. IC51-311 (NCT00595309), followed 199 subjects from IC51-309, was ongoing and investigating the effects of a booster dose of 6 µg of Ixiaro[®] administered 15 months after the initial vaccination. A final trial, IC51-314 (NCT00776230), intended to recruit 300 subjects to assess the immunogenicity of a commercial batch of Ixiaro[®] up to 24 months post-filing. At the time of writing, there were no data available from any of these trials.

Ixiaro[®] has also been studied as a pediatric vaccine and has been shown to prevent encephalitis in childhood.⁴⁸ Analysis of phase II data suggests that a half-dose given to young children (1 to 3 years of age) was highly immunogenic with a safety profile comparable to that of adults taking the full adult dosage. Phase III clinical trials in India are planned to begin in 2009.⁴⁸

Safety, tolerability, side effects and contraindications: recipient satisfaction/acceptability

The phase III randomized, double-blind, placebo-controlled clinical trial, IC51-302 comprehensively assessed the safety and tolerability of Ixiaro[®] in healthy volunteers ($n = 2675$) for up to 2 months after the last vaccination.⁴² Reported adverse effects were similar in recipients of Ixiaro[®] or placebo, and varied in severity from mild to moderate. A total of 17 volunteers, 12 (0.6%) given Ixiaro[®] and 5 (0.8%) given placebo, withdrew from the trial because of treatment-associated adverse effects. In the Ixiaro[®] group, two of these events were considered severe in intensity (gastroenteritis and rash). For those who completed the trial, 12.7% of Ixiaro[®] recipients and 12.2% of placebo recipients reported medically attended adverse events after immunization, of which headache (0.9% and 1.1% for Ixiaro[®] or placebo recipients respectively) and influenza-like illness (1% and 0.9% for Ixiaro[®] or placebo respectively) were the most common. Any adverse effects (medically attended or not) were reported in 58.9% of Ixiaro[®] recipients and 56.6% for those given

placebo, of which headache, again, was the most common (28% and 26.3% for Ixiaro® or placebo, respectively). Other reported adverse effects were myalgia (15.5% and 15.6% for Ixiaro® or placebo, respectively), influenza-like illness (12.4% and 11.9%) and fatigue (11.4% and 11.7%). Local symptoms in both groups were more likely to be reported immediately after vaccination and decreased thereafter. In the first days after vaccination, the most commonly reported adverse events were arm pain and tenderness (10% to 20% in both groups). There were no deaths throughout the trial period and no signs of acute allergic reactions. Thus the frequency of adverse event reporting was very similar in recipients of either Ixiaro® or placebo, and Ixiaro® was reported to be safe. Although there were several instances of fever reported in subsequent clinical trials of Ixiaro®, adverse events were similar to those reported in IC51-302.^{40,42,44}

Data from the long-term follow up clinical trial (IC51-303) reported similar frequencies of mild or serious adverse events in recipients of Ixiaro®, JE-VAX® or placebo, with the most common adverse events being nasopharyngitis, headache or flu-like symptoms.⁴³ Ixiaro® was also safe and well tolerated in infants, and could be safely administered concomitantly with a hepatitis vaccine.⁴⁶

The European Medicines Agency Public Assessment Report contains a summary of all the clinical trial data available at the time of filing for licensure.⁴⁹ The following safety information was derived from 3558 volunteers who had received Ixiaro® in the course of clinical trials to date: That about 40% of treated subjects could expect to experience adverse reactions, usually within the first three days after vaccination. These adverse reactions are usually mild and disappear within a few days. The most commonly reported adverse reactions include headache or myalgia occurring in approximately 20% and 13% of subjects, respectively. Adverse reactions are listed according to the following frequencies: very common: $\geq 1/10$; common: $\geq 1/100$ to $< 1/10$; uncommon: $\geq 1/1,000$ to $< 1/100$; rare: $< 1/10,000$ to $< 1/1,000$; very rare: $< 1/10,000$, not known (cannot be estimated from the available data). The vaccine is recommended for adults only as it has not been tested in children or adolescents, and it should be avoided during pregnancy or lactation as there is only limited data available and its effects have not been fully investigated.

Discussion: the role of Ixiaro® in the prophylaxis of JE

Earth's ecosystem is struggling to cope with the demands of an inexorably increasing population and its demands for food,

energy and other resources. Nowhere is this more apparent than in southern and eastern Asia, an area which currently supports approximately half the world's population. The pressure to feed more mouths as more and more people crowd into cities and urbanization becomes a necessity instead of a luxury, means that more and more of the countryside in southern and eastern Asia is being irrigated to produce rice, and pig breeding is becoming more and more widespread. With these conditions – standing water, waterfowl, and pigs juxtaposed with farmers and their families – it is unsurprising that mosquito-borne diseases such as JE are on the rise, except in those southern and eastern Asian countries which have the resources, infrastructure and economy which can support extensive surveillance and vaccination programs. In large part this is because other measures against mosquitoes such as controlling their breeding or measures to limit human (or pig) exposure to mosquitoes have proven either expensive, difficult to implement, or infeasible. Thus vaccination has proven the most effective means of combating JEV – and other vector borne diseases for which vaccines are available.

Historically, China and Japan have been the main producers of JE vaccines. China currently manufactures a live attenuated vaccine which is available in a limited number of other Asian countries only. The vaccine was produced in a relatively uncharacterized and uncontrolled primary animal cell culture system (PHKs) though the manufacturers are transitioning to a much more well defined and therefore acceptable Vero cell system. The vaccine is very effective – but has not been licensed internationally, and there is no current intention to do so. Japan's vaccine was available internationally – on a named individual basis only. In part this was due to concerns over the production of an inactivated virus vaccine made by propagating virus in mouse brains, and its potential to cause neuronal disease as a result of residual contaminating mouse brain protein, albeit rarely. There were also well documented reports of allergic reactions to the gelatin stabilizer the vaccine contained, especially it seemed in international visitors to the area who thought to protect themselves against JE by taking the vaccine. Following a highly publicized case of acute disseminated encephalomyelitis in 2005, the manufacturers ceased production of the vaccine in 2007 and consequently vaccine stocks are rapidly depleting for both the local populace as well as international visitors to the area. Fortunately, the US military which has a large number of personnel in the area on extended tours of duty and therefore had an interest in protecting its members against endemic diseases, began developing a JE vaccine which would meet stringent regulatory requirements

amongst which were the need to demonstrate the absence of adventitious agents, absence of tumorigenicity and absence of reverse transcriptase activity. This was the inactivated Vero cell-derived virus vaccine which was licensed in by InterCell AG in 2003 and 2004, and is now known as Ixiaro®. The results from the clinical trials described above have determined the vaccine's safety and tolerability profiles and have demonstrated Ixiaro®'s non-inferiority compared with JE-VAX®. Over the last year, these data have contributed to Ixiaro®'s licensing in a number of countries including the US, Europe and Australia.

Clearly the vaccine has been designed for military use as well as for visitors to JE-endemic regions.^{26–28} This market could provide a steady income stream; in 2003, it was estimated that InterCell may be able to sell some 550,000 doses of Ixiaro®, generating income of €150 million⁵⁰ though as the numbers of tourists who visit the area have undoubtedly increased in the intervening years, so too will the numbers of doses they expect to sell and consequently these estimates will have increased in the interim. There is little competition in the market at present although Ixiaro® may have competition in the future if ChimeriVax™-JE⁵¹ (in development by Acambis plc, under license from St. Louis University), which is based on the highly effective yellow fever 17D vaccine, achieves licensure. However, in recent years there have been reports in the medical literature citing cases of viscerotropic disease associated with the use of yellow fever 17D and 17D-based vaccines^{52,53} and the effects of these serious adverse events on the development of products based on the yellow fever 17D vaccines are unclear at present. Other potential competition is in development including Bavarian Nordic's MVA-BN-JE which is in preclinical testing, and a DNA vaccine which has been tested in non-human primates.⁵⁴ Japanese manufacturers are also developing inactivated vaccines based on the Beijing 1 strain of virus propagated in Vero cells, as replacement vaccines for the mouse brain derived vaccine, production of which ceased some years ago. Thus Ixiaro® would appear to have the market to itself for some years. Given that the side effects and adverse events associated with Ixiaro® are generally mild and the risk of infection is low, risk/benefit considerations may tip the balance and it would be of little surprise if the current recommendation for the vaccination of visitors to the areas where JEV is common was revised to recommend that all visitors, irrespective of their length of stay, receive Ixiaro®.

An area where Ixiaro® may find another market is in the pediatric population. A phase II trial showed that Ixiaro®

was both safe and well tolerated in children, almost 100% of whom seroconverted with protective anti-JEV titers. The results of a planned phase III trial of Ixiaro® in pediatric cohorts will determine if Ixiaro® is as safe and effective as a pediatric vaccine as it appears to be in adults.

Note added in proof

Data from the pediatric phase II trial mentioned above has recently been published. Sixty Indian children between 1 and 3 years of age were randomized to 1 of 3 groups given either a) 3 µg or b) 6 µg of Ixiaro® on days 0 and 28, or c) JenceVac™, a generic version of JE-VAX® given by the standard 3-dose schedule. Twelve subjects reported 13 adverse events (fever, injection site tenderness, skin lesion or rash) but the incidence of adverse events was similar in all 3 groups of vaccinees. The immunogenicity of the vaccines was assessed by PRNT assay of serum harvested on days 0, 28 and 56. There were no significant differences in the GMTs elicited by the vaccines at any day of testing and by 56 days after the initial immunization, over 95% of Ixiaro® recipients (and 91% of JenceVac™ recipients) had seroconverted. Thus the safety and immunogenicity data support the use of a 3 µg dose of Ixiaro® in subsequent clinical trials of the vaccine in pediatric populations.

Kaltenböck A, Dubischar-Kastner K, Schuller E, Datla M, Klade CS, Kishore TS. Immunogenicity and safety of IXIARO® (IC51) in a Phase II study in healthy Indian children between 1 and 3 years of age. *Vaccine*. 2009;doi:10.1016/j.vaccine.2009.10.024.

Disclosure

The author discloses no conflicts of interest.

References

1. American Academy of Pediatrics. Arboviruses. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. *Red Book: 2009 Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009;214–220.
2. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet*. 2008; 371(9611):500–509.
3. Tolle MA. Mosquito-borne diseases. *Curr Probl Pediatr Adolesc Health Care*. 2009;39(4):97–140.
4. van den Hurk AF, Ritchie SA, Mackenzie JS. Ecology and geographical expansion of Japanese encephalitis virus. *Annu Rev Entomol*. 2009; 54:17–35.
5. Solomon T, Vaughn DW. Pathogenesis and clinical features of Japanese encephalitis and West Nile virus infections. *Curr Top Microbiol Immunol*. 2002;267:171–194.
6. Kollaritsch H, Paulke-Korinek M, Dubischar-Kastner K. IC51 Japanese encephalitis vaccine. *Expert Opin Biol Ther*. 2009;9(7):921–931.
7. Wilder-Smith A. Japanese encephalitis: is there a need for a novel vaccine? *Expert Rev Vaccines*. 2009;8(8):969–972.
8. Marfin AA, Barwick Eidex RS, Kozarsky PE, Cetron MS. Yellow fever and Japanese encephalitis vaccines: indications and complications. *Infect Dis Clin N Am*. 2005;19(1):151–168.

9. Erlanger TE, Weiss S, Keiser J, Utzinger J, Wiedenmayer K. Past, present and future of Japanese encephalitis. *Emerg Infect Dis*. 2009;15(1):1–7.
10. Beasley DWC, Lewthwaite P, Solomon T. Current use and development of vaccines for Japanese encephalitis. *Expert Opin Biol Ther*. 2008;8(1):95–106.
11. Das S, Basu A. Japanese encephalitis virus infects neural progenitor cells and decreases their proliferation. *J Neurochem*. 2008;106(4):1624–1636.
12. Ghoshal A, Das S, Ghosh S, et al. Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. *Glia*. 2007;55(5):483–496.
13. [No authors listed]. Japanese encephalitis vaccines. *Wkly Epidemiol Record*. 2006;81(34/35):331–340.
14. Craven RB, Monath TP, Poland JD, Cropp CB. Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in US inhabitants. *J Infect Dis*. 1990;161(5):878–882.
15. Robinson P, Ruff T, Kass R. Australian case-control study of adverse reactions to Japanese encephalitis vaccine. *J Travel Med*. 1995;2(3):159–164.
16. Berg SW, Mitchell BS, Hanson RK, et al. Systemic reactions in US Marine Corps personnel who received Japanese encephalitis vaccine. *Clin Infect Dis*. 1997;24(2):265–266.
17. Plesner AM, Ronne T. Allergic mucocutaneous reactions to Japanese encephalitis vaccine. *Vaccine*. 1997;15(11):1239–1243.
18. Plesner AM, Ronne T, Wachmann H. Case-control study of allergic reactions to Japanese encephalitis vaccine. *Vaccine*. 2000;18(17):1830–1836.
19. Sakaguchi M, Inouye S. Systemic allergic reactions to gelatin included in vaccines as a stabilizer. *Jpn J Infect Dis*. 2000;53(5):5189–5195.
20. Ohtaki E, Murakami Y, Komori H, Yamashita Y, Matsuishi T. Acute disseminated encephalomyelitis after Japanese B encephalitis vaccination. *Pediatr Neurol*. 1992;8(2):137–139.
21. Plesner A-M, Arlien-Soborg P, Herning M. Neurological complications to vaccination against Japanese encephalitis. *Eur J Neurol*. 1998;5(5):479–485.
22. Takahashi H, Pool V, Tsai TF, Chen RT, The VAERS Working Group. Adverse events after Japanese encephalitis vaccination: review of post-marketing surveillance data from Japan and the United States. *Vaccine*. 2000;18(26):2963–2969.
23. Huynh W, Cordato DJ, Kehdi E, Masters LT, Dedousis C. Post-vaccination encephalomyelitis: Literature review and illustrative case. *J Clin Neurosci*. 2008;15(12):1315–1322.
24. Macdonald W, Tink A, Ouvrier R, et al. Japanese encephalitis after a two-week holiday in Bali. *Med J Aust*. 1989;150(12):334–337.
25. Wittesjö B, Eitrem R, Niklasson B, Vene S, Mangiafico JA. Japanese encephalitis after a 10-day holiday in Bali. *Lancet*. 1995;345(8953):856–857.
26. Buhl MR, Lindquist L. Japanese encephalitis in travelers: review of cases and seasonal risk. *J Travel Medicine*. 2009;16(3):217–219.
27. Burchard GD, Caumes E, Connor BA, et al. Expert opinion on vaccination of travelers against Japanese encephalitis. *J Travel Medicine*. 2009;16(3):204–216.
28. Hatz C, Werlein J, Mutsch M, Hufnagel M, Behrens RH. Japanese encephalitis: defining risk incidence for travelers to endemic countries and vaccine prescribing from the UK and Switzerland. *J Travel Medicine*. 2009;16(3):200–203.
29. Inactivated Japanese encephalitis virus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 1993;42(RR-1):1–15.
30. Kim HS, Yoo WD, Kim SO, et al. inventors; Cheil Jedang Corp, USA Secretary of the Army, assignees. Attenuated Japanese encephalitis virus adapted to Vero cell and a Japanese encephalitis vaccine. US Patent US6309650. 2001 October 30.
31. Kim HS, Yoo WD, Kim SO, et al. inventors; Cheil Jedang Corp, assignee. An attenuated Japanese encephalitis virus adapted to Vero cell and a Japanese encephalitis vaccine. International Patent Application WO/99/11762. 1999 March 11.
32. Kim HS, Yoo WD, Kim SO, et al. inventors; Cheil Jedang Corp, assignee. EP-01604685: An attenuated Japanese encephalitis virus. CJ Corp, Walter Reed Institute of Research European Patent Application EP-1604685. 1998 August 25.
33. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases. 2006 September. URL: <http://www.fda.gov/CBER/gdlns/vaccsubstrates.pdf>.
34. Montagnon BJ, Vincent-Falquet JC. Experience with the Vero cell line. *Dev Biol Stand*. 1998;93:119–123.
35. Eckels KH, Yong-Xin Y, Dubois DR, Marchette NJ, Trent DW, Johnson AJ. Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA14–14–2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use. *Vaccine*. 1988;6(6):513–518.
36. Srivastava AK, Putnak JR, Lee SH, et al. A purified inactivated Japanese encephalitis virus vaccine made in Vero cells. *Vaccine*. 2001;19(31):4557–4565.
37. Aimanianda V, Haensler J, Lacroix-Desmazes S, Kaveri SV, Bayry J. Novel cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants. *Trends Pharmacol Sci*. 2009;30(6):287–295.
38. Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. *Nat Rev Immunol*. 2009;9(4):287–93.
39. Van Gessel Y, Putnak R, Gettayacamin M, et al. Passive transfer of human antibodies against a new Japanese encephalitis virus vaccine protects mice against lethal dose of virus. *Am J Trop Med Hyg*. 2006;75(5 Suppl):282.
40. Lyons A, Kanasa-athan N, Kuschner RA, et al. A phase 2 study of a purified, inactivated virus vaccine to prevent Japanese encephalitis. *Vaccine*. 2007;25(17):3445–3453.
41. Tauber E, Kollaritsch H, Korinek M, et al. Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomized controlled trial. *Lancet*. 2007;370(9602):1847–1853.
42. Tauber E, Kollaritsch H, von SF, Lademann M, et al. Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine. *J Infect Dis*. 2008;198(4):493–499.
43. Schuller E, Jilma B, Voicu V, et al. Long-term immunogenicity of the new Vero cell-derived, inactivated Japanese encephalitis virus vaccine IC51. Six- and 12-month results of a multicenter follow-up phase 3 study. *Vaccine*. 2008;26(34):4382–4386.
44. Schuller E, Klade CS, Heinz FX, et al. Effect of pre-existing anti-tick-borne encephalitis virus immunity on neutralizing antibody response to the novel, Vero cell-derived, inactivated Japanese encephalitis virus vaccine IC51. *Vaccine*. 2008;26(48):6151–6156.
45. Schuller E, Klade CS, Wölfl G, Kaltenböck A, Dewasthaly S, Tauber E. Comparison of a single, high-dose vaccination regimen to the standard regimen for the investigational Japanese encephalitis vaccine, IC51: a randomized, observer-blind, controlled Phase 3 study. *Vaccine*. 2009;27(15):2188–93.
46. Lehner C, Eder G, Formica A, et al. Safety and immunogenicity of concomitant vaccination with IC51 and hepatitis A vaccine in healthy subjects. A single-blind randomized controlled phase 3 study. *Am J Trop Med Hyg*. 2007;77(Suppl 5):36.
47. Kaltenböck A, Dubischar-Kastner K, Eder G, et al. Safety and immunogenicity of concomitant vaccination with the cell-culture based Japanese Encephalitis vaccine IC51 and the hepatitis A vaccine HAVRIX1440 in healthy subjects: A single-blind, randomized, controlled Phase 3 study. *Vaccine*. 2009;27(33):4483–4489.
48. Intercell Annual Report 2008. URL: http://www.intercell.com/uploads/media/2008_Annual_Report.pdf
49. European Public Assessment Report for authorized medicinal products for human use. Available from: <http://www.emea.europa.eu/humandocs/Humans/EPAR/Ixiaro/Ixiaro.htm>
50. Intercell Annual Report 2003. URL: http://www.intercell.com/uploads/media/2003_Annual_Report.pdf

51. Monath TP, Guirakhoo F, Nichols R, et al. Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax™-JE): phase II 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):1213–1230.
52. Lindsey NP, Schroeder BA, Miller ER, et al. Adverse event reports following yellow fever vaccination. *Vaccine*. 2008;26(48):6077–82.
53. Whittembury A, Ramirez G, Hernández H, et al. Viscerotropic disease following yellow fever vaccination in Peru. *Vaccine*. 2009;Epub Aug 11.
54. Bharati KB, Rani R, Vrati S. Evaluation of Japanese encephalitis virus DNA vaccine candidates in rhesus monkeys [Macaca mulatta]. *Vaccine*. 2009;27(1):10–16.

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