Drugs of the Future 2009, 34(6): 458-467

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.6.1383739

IC51

Japanese Encephalitis Vaccine

IXIARO®
JESPECT®

Japanese encephalitis vaccine based on inactivated and attenuated purified virus, containing the strain SA_{14}-14-2 produced in Vero cells, adjuvanted with aluminium hydroxide and delivered in prefilled syringes

EN: 342877

ABSTRACT

Japanese encephalitis (JE), a mosquito-borne arboviral infection, is the leading cause of viral encephalitis in Asia. An annual occurrence of approximately 50,000 sporadic and epidemic cases has been estimated. Vaccines are available for active immunization. A new purified, inactivated JE virus vaccine (IC51) has been developed, which is manufactured in a Vero cell culture substrate. Since early 2009, the vaccine is licensed in Australia, the U.S. and Europe. A series of successful phase III studies have been published, indicating that this vaccine will be a modern approach to active immunization against JE.

BACKGROUND

Japanese encephalitis virus (JEV) is a *Flavivirus*, which are enveloped, positive-sense, single-stranded RNA viruses that are largely vector-borne (1, 2). After an infectious mosquito bite to a mammal host, viral replication occurs locally and in regional lymph nodes. The incubation period for Japanese encephalitis (JE) is 5-15 days. Central nervous system invasion likely occurs from the blood (3-5). The vast majority of infections are not apparent and only 1 in 25-1,000 infections results in symptomatic illness (6). The main clinical manifestation of illness is encephalitis and a progressive decline in alertness, which eventually leads to coma of varying degree. A majority of patients become totally unresponsive or responsive only to pain. Generalized weakness and changes in tone, especially hypertonia and hyperreflexia, are the most common motor abnormalities (7). Recrudescent symptoms several months after the initial disease have been described (8). Approximately 33% of symptomatic cases are fatal, some occurring after a brief prodromal phase and fulminant course lasting a few days. Others follow a more protracted route into coma. Overall, approximately one-third of patients with encephalitis exhibit serious residual neurological disabilities (9-13). Principal sequelae include memory loss, impaired cognition, behavioral disturbances, convulsions, motor weakness or paralysis, and abnormalities of tone and coordination.

Laboratory confirmation, principally by serology, should be attempted to prove the infection (14). Unfortunately, the sensitivity of laboratory methods is not adequate to guarantee proper diagnosis in all patients. The most widely used diagnostic method is IgM capture ELISA (immunogloblulin M capture enzyme-linked immunosorbent assay) (15-17). Specific IgM can be detected in cerebrospinal fluid (CSF) and/or serum in approximately 75% of patients within the first 4 days after the onset of illness, and nearly all patients are positive 7 days after onset. A specific diagnosis can also be confirmed by demonstrating fourfold or greater changes in antibody titer by conventional serological procedures, e.g., hemagglutination inhibition, complement fixation, immunofluorescence, ELISA or neutralization. For evaluating protective immunity against JEV infection after vaccination, plaque reduction neutralization tests (PRNT) are frequently used and considered the gold standard for confirming induction of protective immunity (18). A PRNT titer of 1:10 and higher is assumed as a surrogate parameter for protection (18, 19). Current treatment of JE is supportive only (15).

JEV is the leading cause of viral encephalitis in Asia and almost 60% of the world's population, about 3 billion people, are living in areas endemic for the virus (15, 20). In highly endemic areas, incidence rates of the disease are up to 10 per 100,000 population and year (15, 21). Each year, 30,000-50,000 symptomatic cases of JE are reported from Asia and Australia, accounting for at least 10,000 deaths and 15,000 cases of neuropsychiatric sequelae (21). However, underreporting is common and estimates are as high as up to 175,000 symptomatic cases of JE per year (22). By the age of 15 years, almost all people living in endemic regions have been infected with JEV (21). The majority of patients are reported from China, the Indian subcontinent and Southeast Asia (23-30). In the last few decades, the disease has been spreading south and west, reaching Pakistan and also Pacific regions. Recently, JE has also emerged to the north of the Australian subcontinent, in particular in the Torres Strait islands (31). JE is principally a disease of rural agricultural areas, where vector mosquitoes proliferate in close association with pigs, wading birds and ducks, the principal vertebrate amplifying hosts. Humans and horses are incidental hosts and may become ill

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after infection (15). Viral persistence in vertebrate hosts, such as bats or reptiles, and annual reintroduction of the virus through migrations of birds or wind-borne mosquitoes serve as important mechanisms for the occurrence of JE in temperate regions.

Culex tritaeniorhynchus is the principal JE vector in most areas of Asia (15, 32). These and other principally culicine species use ground pools and especially flooded rice paddies as larval habitats. Agricultural practices, and specifically the irrigated cultivation of rice, exert a profound influence on vector bionomics. A single rice paddy may produce more than 30,000 adult mosquitoes in a single day (15, 33). These enhanced agricultural cultivations, and consequently expanding habitats for the mosquitoes, provide one key reason for the expansion of JEV (21, 34). Although the risk for acquiring JE is highest in rural areas, conditions that permit enzootic viral transmission exist within or at the periphery of many Asian cities. For example, JE cases in Taiwan are reported principally from Taipei and surrounding areas; in Vietnam, the incidence of JE is highest in and near Hanoi; and cases are frequently reported from suburban areas of major cities such as Bangkok, Beijing and Shanghai (35-39). Sporadic reports of cases and outbreaks from India, Bali, Singapore and Hong Kong also attest to the possibility of enzootic viral transmission near highly developed urban areas. Periodic epidemic outbreaks have been observed every 2-15 years in many Asian countries (20).

Since several endemic countries implemented rigorous vaccination programs that led to a near elimination of human disease, official reports might not mirror the actual risk of infection (40, 41). China is a leading example of the effective implementation of vaccination programs against JE (15, 16). In 1990, 2.5 cases of JE per 100,000 population occurred, and following widespread intervention, 0.5 JE cases per 100,000 population occurred in 2004 (16).

No systematic surveillance data are available on JE cases in travelers. Data from published case reports, informal surveillance of laboratories and personal communications with ministries of health and travel clinics indicate that the risk for JE among foreign nationals in Asia deemed to be underreported. Infection rates in unimmunized American, Australian and British soldiers exposed in Asia have been reported to range from 0.1 to 2.1/10,000 per week (15). Obviously, the risk for acquiring JE during travel is highly variable and depends on the destination, season of travel and activities of the individual. Recent publications document case series in travelers with few excursions into rural areas while on short holidays in endemic areas such as Bali, Hong Kong, Vietnam, Thailand and others (15, 35, 36, 38, 39). Data from Finland and Sweden suggest an incidence of symptomatic JE in travelers of 1:275,000 and 1:400,000, respectively (39, 42).

Apart from exposure prophylaxis against mosquito bites, vaccination is the only preventive measure against JE. The virus exists as one serotype (43); however, natural diversity of JE viral strains has been demonstrated, with minor antigenic differences among strains and in biological characteristics (15, 44). The Nakayama strain of JEV, isolated from spinal fluid of a human case in 1935 and maintained by continuous mouse brain passage, was initially the principal strain used in mouse brain-derived vaccines produced throughout Asia (45). Because of the expansion of epidemic JE to wider areas of Asia, crossimmunization studies were repeated with strains from diverse areas of Asia. Among those, Beijing-1, and later SA_{14}-14-2, were chosen as seed strains due to their good immunogenicity in mice and men. Mouse brain-derived inactivated JE vaccines were produced as early as the 1930s in Russia and Japan (15). Successive refinements of the mouse brain vaccine were introduced by Japanese research institutes, which afforded the purified vaccine that has been used in Japan until very recently (15, 45).

Several vaccines (live and inactivated) are being used in preventive programs: 1) inactivated vaccines derived from mouse brain; 2) inactivated vaccines cultivated in primary hamster kidney (PHK) cells; and 3) live attenuated vaccines cultivated in PHK cells and based on the Chinese virus strain SA_{14}-14-2 (46).

The inactivated mouse brain-derived vaccine is a formalin-inactivated whole virus vaccine containing either the Nakayama or the Beijing-1 strain. The vaccine was first developed in Japan (46) and is regionally produced in many countries, including South Korea, Taiwan, India and Russia. It was produced by BIKEN (Japan) and has been distributed by Sanofi Pasteur as JE-VAX® in the U.S. (46, 47). This vaccine has been associated with a moderate frequency of local and mild systemic adverse reactions (48-51). Local effects like tenderness, redness and swelling have been reported in approximately 20% of vaccinees. Systemic side effects like fever, headache, malaise, rash, chills, dizziness, myalgia, nausea, vomiting and abdominal pain were observed in 10% of vaccinees. Since the BIKEN vaccine is manufactured from neural tissue, acute disseminated encephalomyelitis and other neurological side effects have been, and are, a concern (52, 53). The Committee on Side Reactions of the Ministry of Health and Welfare in Japan found no documented occurrence of allergic encephalitis in 38,384 vaccinees between 1957 and 1965 (49). Further intense surveillance of JE vaccine-related complications in Japan during 1965-1973 revealed neurological events like encephalitis, encephalopathy, seizures and peripheral neuropathy in 1-2.3/1,000,000 vaccinees. However, a causal relationship between the JE vaccine and temporally related neurological events has been challenged (24).

An apparently new pattern of adverse reactions has been reported since 1989, mostly among travelers vaccinated in Australia, Europe and North America (51, 54, 55). The reactions have been characterized by urticaria, often in a general distribution, angioedema, respiratory distress and collapse due to hypotension. Although most patients were treated successfully with antihistamines or steroids, some had to be hospitalized. The interval between vaccination and onset of symptoms was an important feature in these reactions. Reactions after the first JE vaccine dose occurred after a median of 12 h following vaccination and 88% of reactions occurred within 3 days (54, 55). The interval between administration of a second dose and onset of symptoms was generally longer (median of 3 days), possibly as long as 2 weeks. Reactions occurred after a second or third dose, with preceding doses being uneventful. Therefore, based on the data of one prospective study, similar reaction rates for first and second doses have been implicated (24).

The incidence of the described side effects varied markedly depending on the circumstances of vaccine administration and surveillance. Rates of 50-104/10,000 vaccinees have been reported from travel clinics in Australia and Canada. National surveillance data from Denmark, Australia, the U.K. and Sweden implied 10-fold lower rates

of approximately 0.7-12/10,000 (24). A trial in more than 500 German travelers showed the occurrence of side effects in 54% of all vaccinees (56). However, the majority of the reported side effects consisted of harmless local (mild to moderate swelling and local pain) and short (duration: average of 4.1 days) systemic reactions. None of the travelers experienced long-lasting adverse reactions or serious anaphylactic symptoms. Most experts implicate the porcine gelatin stabilizers, included in the BIKEN, JE-VAX® and South Korean Green Cross formulations, to be responsible for these severe adverse effects. Due to the above-mentioned adverse reactions, the World Health Organization (WHO) proposed the development of a new generation of JE vaccines (22, 57). These safety concerns also led to the suspension of routine vaccination with the mouse brain-derived inactivated JE vaccines in Japan in May 2005 (21) and a halt of distribution of JE-VAX® in the U.S.

An inactivated vaccine used most frequently, with about 70 million doses administered every year in China, was cultivated in PHK cell cultures and contained the P3 strain. Although the vaccine contains gelatin and sucrose, it is well tolerated. It also induces good immunological memory (15). The old PHK-derived P3 vaccine was available in China only and was recently replaced by the Vero cell-derived P3 killed vaccine (58). This vaccine is not available for the market outside China.

An alternative to the mouse brain method has been presented by Chinese scientists. A live attenuated vaccine was developed that was derived from JEV strain SA_{14}-14-2, which was passed through mice and is produced in primary baby hamster cells (15). Trials with this vaccine appear to confirm its safety and high immunogenicity (15, 59), and the vaccine has been administered to over 100 million children during campaigns in China. According to the WHO, the live attenuated SA_{14}-14-2 vaccine constituted more than 50% of the global production of all JE vaccines in 2005 (21). Its low cost per dose ($0.03 compared with $2.30 for the BIKEN vaccine) renders it useful for mass vaccination. However, doubts regarding its safety still inhibit widespread use outside China and some other Asian countries (e.g., Nepal, India, South Korea and Thailand) (60). Since it is a live attenuated vaccine, the theoretical risk of mutation of vaccine strains to pathogenic strains remains (26). Recently, production and control standards have been upgraded in order to acquire wider international licensure for this vaccine (61). Although not WHO prequalified, the Chinese manufacturer Chengdu Institute of Biological Products has reportedly complied with WHO production standards since 2006 (15). The vaccine was proven to be safe and effective in children (62), providing 80-96% protection after a single dose and an efficacy of 97.5% after two doses 1 year apart (60), and a Nepalese field study revealed an efficacy of 96.2% for a single dose for 5 years (63, 64). However, there is apparently no intention to license this vaccine in Europe or the U.S.

Candidate JE vaccines are being developed, as shown in Table I. They include the live attenuated YFV-17D/JEV vaccine developed by Acambis (58). This approach uses a genetically modified organism. A chimeric YF/JEV was constructed by insertion of the premembrane and envelope (prME) genes of an attenuated human vaccine strain (SA_{14}-14-2) of JEV between the core and nonstructural (NS) genes of a YF 17D infectious clone (18, 65, 66). Phase II studies have shown a 94% seroconversion rate in 87 vaccinated subjects following a single vaccination (67). Currently, recruitment for phase III studies in Thailand is ongoing. This vaccine shows an interesting potential, but obviously more data will be needed before full assessment can be made.

Following the WHO recommendations, a JE vaccine with an altered immunogenicity and safety profile is desired. IC51 has been developed by the Austrian company Intercell AG and was approved for adults in early 2009 by the FDA and the European Medicines Agency (EMEA) under the trade name IXIARO® and by Australia's Therapeutic Goods Administration (TGA) under the trade name JESPECT®. IC51 is a purified, formalin-inactivated, whole-virus JE vaccine based on the strain SA_{14}-14-2 and cultivated in Vero cells (68). The vaccine is manufactured according to GMP. One dose contains 6 µg of inactivated and purified SA_{14}-14-2 virus adsorbed to 0.1% aluminum hydroxide.

The wild-type parental virus SA14 was isolated from a pool of *Culex pipiens* larvae from Xian, China, following 11 passages in mouse brain. Harvesting of the strain SA_{14}-14-2 was performed through an empirical process of serial passage performed in China in PHK cells.

Table I. *Japanese encephalitis vaccines for travelers: a selected overview.*

Name	BIKEN/JE-VAX®	SA_{14}-14-2 vaccine	IC51	ChimeriVax™-JE	MVA-BN-JE
Manufacturer	BIKEN, Japan; Sanofi Pasteur, USA; Green Cross Corp., South Korea	Chengdu Institute of Biological Products, China	Intercell, Austria	Acambis, U.K.	Bavarian Nordic, Germany
Current status	Most manufacturers ceased production	No WHO prequalification	Phase III completed, licensure in Australia, U.S. and Europe since early 2009	Phase III in Asia, Australia and U.S.	Preclinical
Type	Killed	Live	Killed	Live attenuated	Live attenuated
Virus	Nakayama	SA_{14}-14-2	SA_{14}-14-2	SA_{14}-14-2 and YF 17D infectious clone	Vaccinia Ankara vector
Dosing	Three doses on days 0, 7 and 28	Single dose	Two doses on days 0 and 28	Single dose	Undetermined

This strain was given the designation SA14 clone 14-2 (also designated SA_{14}-14-2). Forty-five nucleotide differences, resulting in 15 amino acid substitutions, were detected by comparing sequences of the original SA14 and the strain SA_{14}-14-2 genomes. At the Walter Reed Army Institute of Research (WRAIR), the SA14 clone 14-2 was first adapted to primary canine kidney (PDK) cells and then to Vero cells. Attenuated JE vaccine SA_{14}-14-2 was previously passaged eight times in certified PDK cells and then passaged five times in certified Vero cells and used as the master seed for the inactivated vaccine (69).

PRECLINICAL PHARMACOLOGY

Immunogenicity studies with the inactivated vaccine were first performed in rats and rabbits. Results showed a strong antibody response in both species. Preclinical assays for demonstration of active protection were done at the WRAIR by challenge studies in mice. Groups of 10 weanling, outbred mice were vaccinated with graded doses of the alum-adjuvanted, purified, inactivated JE vaccine IC51 or with JE-VAX®. Animals were boosted on day 14 and challenged with JEV on day 21 with approximately 1,000 times the LD_{50}. The LD_{50} for IC51 was calculated to be 2.6 ng (95% confidence interval [CI]: 0.2-7.6 ng) and 1.5 ng for JE-VAX® (95% CI: 0.6-3.7 ng) (69). Preclinical studies also showed that PRNT titers can serve as surrogate markers for protection. Passive immune transfer from human sera with high postvaccination PRNT titers was able to protect mice from lethal challenge with SA14 and the non-genotype 3 JE strain KE-093 (after immunization with IC51 in the pivotal phase III immunogenicity study) (29). This vaccine has induced an in vivo titer of ≥ 1:10 in mice in previous immunizations (70). The 1:10 threshold has also been accepted by expert forums (71). Using human immune sera from the pivotal immunogenicity study (18) and from a study following up long-term immunogenicity in those subjects (72), IC51 was shown to be able to elicit a broad neutralizing antibody response in vitro against a variety of JEV strains (SA14, Nakayama, Beijing, P-20778) (70).

SAFETY

A series of studies and one pooled analysis of reactogenicity data have been published (18, 72-75). Of 4,715 subjects who were included in the 6-month pooled safety analysis (74), 54.1% of those vaccinated with IC51 (n = 3,558) reported local reactions after vaccination. In the placebo vaccine groups (n = 657), local reactions were reported in 56.1%, and for 61.1% in the JE-VAX® group (n = 435). Severe local symptoms were reported in 3.2%, 3.1% and 13.8%, respectively, giving significant evidence for less favorable local tolerance of JE-VAX®. IC51 and placebo (phosphate-buffered saline with 0.1% aluminum hydroxide) showed comparable local tolerability.

Systemic tolerance was generally excellent in all reported trials. The percentage of subjects reporting any adverse event following immunization was 58.9% in the IC51 group and 56.6% in the placebo group (75). In a head-to-head study with the well-tolerated hepatitis A vaccine Havrix®1440 (control group), IC51 showed a similar safety profile: 47.7% of the subjects reported an adverse event following immunization with Havrix®1440 compared to 41.5% in the IC51 group (76).

The frequency of adverse events following immunization (AEFI) requiring medical attention during the pivotal safety study was 12.7% in the IC51 group (n = 1,993) and 12.2% in the placebo group (n = 657). The most frequently reported AEFIs with medical attention were headache (0.9%), urinary tract infection (0.6%), sinusitis/nasopharyngitis/bronchitis (1.5% combined), influenza-like illness (1.0%), myalgia (0.4%) and (predominantly low-grade) fever (0.3%). Serious adverse events following immunization (SAEFI) occurred in 0.5% for IC51 and 0.9% for placebo. None of the SAEFIs could be attributed to either study medication, in that no striking symptom was observed repeatedly (75). None of the AEFIs assessed as possibly related to vaccination occurred significantly more often in the IC51 group as compared to placebo. Only two cases of transient urticaria were noted 6 days (placebo group) and 8 days (IC51) after vaccination. None of the trials reported any serious allergic reactions. No angioedema was observed and no cases of severe neurological side effects assessed as at least possibly vaccine-related by the investigator were detected. However, as reports from the formerly used JE-VAX® vaccine indicate, severe neurological side effects or life-threatening allergic reactions occur with an incidence of 1-58 x 10^{-4} (13, 58, 75, 77-79). Such rare events may not have been detectable by the number treated so far with IC51.

CLINICAL STUDIES

A total of 10 clinical trials have been completed to date: a phase I study (WRAIR 763), a phase II study (WRAIR 815) and 8 phase III studies. Further studies are ongoing or are planned, in particular regarding long-term immunogenicity, lot shelf-life and vaccination of children. An overview is given in Table II (see also http://clinicaltrials.gov/ct2/results?term=IC51) (80).

The phase I study was designed as a dose- and schedule-finding study. Data from this study did not indicate problems with safety or immunogenicity. Testing of different numbers of vaccinations (two versus three shots; WRAIR 815) and different amounts of antigen (0.4, 2 and 6 µg) led to the selection of higher antigen concentrations (6 and 12 µg) and a two-dose schedule (days 0 and 28). The latter was compared in the first phase II study with a conventional three-dose schedule (days 0, 7 and 28; WRAIR 815). This study showed that two and three vaccination doses of IC51 produced antibodies against JEV for up to 2 years (68). An additional study to evaluate the immunogenicity of a single-shot regimen (6 and 12 µg in comparison with a two-dose 6 µg schedule) was added (76).

In a randomized phase III trial, the immunogenicity and safety of IC51 were compared with JE-VAX® in adults (≥ 18 years). Study participants received two doses of IC51 at a 4-week interval (and placebo on day 7) or three doses of JE-VAX® on days 0, 7 and 28 (18). Four weeks after the second dose of IC51, the seroconversion rate (SCR) was 98% compared to 95% after three doses of JE-VAX® (43). Noninferiority of IC51 in comparison to JE-VAX® was demonstrated for the parameters SCR (at a noninferiority margin of 10% difference) and geometric mean titer (GMT ratio > 1/1.5) (18). Moreover, geometric mean neutralizing antibody titers determined by PRNT on day 56 (4 weeks after the second dose of IC51 or the third dose of JE-VAX®) were 2.3-fold higher in the IC51 group. Geometric mean titer for recipients of the test vaccine was 244 (range: 5-19,783) compared with 102 (range: 5-1,864) for JE-VAX® (ratio: 2.3; 95% CI: 1.97-2.75).

Table II. *IC51 - summary of clinical studies (adapted from Ref. 81) (http://clinicaltrials.gov/ct2/ results?term=IC51).*

Study	Description	Aim (endpoints)	Treatment/ comparator	Amount of antigen	No. subjects planned (actual)	Summary (Ref.)
Phase I						
WRAIR 763	Dose- and regimen-finding	Prim: safety Sec: immune response to IC51, SCR and GMT on day 56	IC51	2 x 0.4 µg 3 x 0.4 µg 2 x 2.0 µg 3 x 2.0 µg	n = 7 (6) n = 7 (5) n = 7 (7) n = 7 (7) Σ = 28 (25)	No SAEs, dose-dependent local reactions, immunogenicity slightly dependent on dose
Phase II						
WRAIR 815	Dose- and regimen-finding	Prim: immune response to IC51; immunogenicity of IC51 vs. JE-VAX®; anti-JEV neutralizing antibody titer at day 56 Sec: safety, immunogenicity until month 24	IC51 JE-VAX®	2 x 6.0 µg 3 x 6.0 µg 2 x 12.0 µg 3 x 1 mL	n = 25 (24) n = 25 (24) n = 25 (25) n = 25 (21) Σ = 100 (94)	95-100% SCR (IC51) vs. 75% SCR (JE-VAX®), good safety profile (68)
Phase III						
IC51-301	Noninferiority of IXIARO® vs. JE-VAX®	Prim: noninferiority of SCR and GMT on day 56 Sec: safety	IC51 JE-VAX®	2 x 6.0 µg 3 x 1 mL	n = 429 (430) n = 429 (437) Σ = 858 (867)	(18)
IC51-302	Safety: serious and medically attended adverse events IXIARO® vs. placebo	Prim: safety	IC51 Placebo	2 x 6.0 µg 2 x 0.5 mL	n = 2,010 (2,012) n = 670 (663) Σ = 2,680 (2,675)	(75)
IC51-303	Long-term immunogenicity and safety	Safety up to month 6	NA	NA	6-Month safety n = 3,920 (3,258) (from IC51-301 and IC51-302)	Ongoing; first part published (72): summarized data (74)
		Prim: SCR at month 24 Sec: SCR and GMT at months 6, 12, 24, 36, 48 and 60; long-term safety	NA	NA	Long-term immunogenicity n = 160 (181)	Ongoing; first part published (72)
IC51-304	Noninferiority of rapid immunization; safety	Prim: SCR on day 56 Sec: SCR and GMT on days 10, 28 and 35; safety	IC51	2 x 6.0 µg vs. 1 x 6.0 µg vs. 1 x 12.0 µg	n = 125 per group Σ = 375 (374)	(73, 84)
IC51-305	Immunogenicity: long-term at 24 months for subjects with positive PRNT results in IC51-304; effect of booster dose 11 and/or 23 months after first vaccination in subjects showing negative PRNT results at month 6 or 12 after primary immunization, respectively; safety	Prim: SCR at month 24 Sec: SCR at month 1 after booster vaccination; safety	IC51	1 x 6.0 µg	n = 375 from IC51-304	(81)

Continued

Table II (Cont.). *IC51 - summary of clinical studies (adapted from Ref. 81) (http: //clinicaltrials.gov/ct2/ results?term=IC51).*

Study	Description	Aim (endpoints)	Treatment/ comparator	Amount of antigen	No. subjects planned (actual)	Summary (Ref.)
Phase III						
IC51-308	Noninferiority of covaccination of IXIARO® with Havrix®1440; safety	Prim: GMT for anti-JEV neutralizing antibody at day 56 and anti-HAV antibody at day 28 Sec: SCR on day 56; GMT, SCR on day 28; safety	IC51 Havrix®1440	2 x 6.0 µg IXIARO® plus Havrix®1440 (1 x 1 mL) vs. 2 x 6.0 µg IXIARO® plus placebo (1 x 1 mL) vs. placebo (2 x 0.5 mL) plus Havrix®1440 (1 x 1 mL)	n = 64 per group Σ = 192 (192)	No interference with immune responses to HAV and JEV vaccine (76)
IC51-309	Equivalence of three IXIARO® batches in terms of immunogenicity	Prim: GMT on day 56 Sec: SCR on day 56; safety (up to 6 months)	IC51 (three batches)	2 x 6.0 µg	n = 208 per group Σ = 624 (639)	All batches high SCRs, completed
IC51-310	Equivalence of three IXIARO® commercial batches	Prim: GMT on day 56 Sec: SCR on day 56; GMT and SCR on day 28; safety and tolerability	IC51 (three batches)	2 x 6.0 µg	n = 384 (389)	All batches equivalent for GMT and SCR, completed
IC51-311	Effect of a booster dose and long-term immunogenicity at 12 months	Prim: SCR at month 12 after booster dose Sec: SCR on day 28 and month 6; GMT on day 28, month 6 and month 12 after booster dose; safety, tolerability	IC51	1 x 6.0 µg	200 (199)	Ongoing
IC51-314	Immunogenicity of a commercial batch of IC51 up to 24 months postfilling	Prim: GMT at day 56 Sec: SCR, GMT on day 28, month 6 and month 12; treatment-emergent adverse events; systemic and local tolerability	IC51	2 x 6.0 µg	300	Ongoing

GMT, geometric mean titer; HAV, hepatitis A virus; JEV, Japanese encephalitis virus; NA, not applicable; Prim, primary objective; PRNT, plaque reduction neutralization test; SAE, serious adverse event; SCR, seroconversion rate; Sec, secondary objective; placebo, phosphate-buffered saline with 0.1% aluminum hydroxide.

A subgroup of the IC51-vaccinated subjects of this study was allocated to long-term follow-up for immunogenicity. First results on long-term immunogenicity are available, showing that at month 6 the IC51-vaccinated subjects had higher SCRs than JE-VAX®-vaccinated subjects (95% vs. 74%) and higher GMTs (84 vs. 34 in the PRNT). At month 12, the SCR of the IC51 subjects was 83% and the GMT remained almost stable at 41. This indicates a typical antibody decline pattern for IC51, with an initial rapid decline followed by an almost stable antibody titer (72).

A study to evaluate the immunogenicity of a single-shot regimen (6 and 12 µg) was added. This study showed a low seroconversion rate at day 28 after the first vaccination (40% and 66%, respectively) (73). IC51 depends on a prime–boost schedule with two vaccinations.

Long-term immunogenicity and the effect of booster vaccination are currently under investigation. Booster doses at 11 and/or 23 months after a full primary schedule or a single dose of 6 or 12 µg will be evaluated, followed by long-term control of GMT and SCR (81).

The concomitant administration of IC51 and an inactivated hepatitis A vaccine was evaluated in a separate study (76). IC51 did not influence the immune response against hepatitis A vaccine (Havrix®1440) and vice versa, and was generally well tolerated.

The influence of pre-existing tick-borne encephalitis (TBE) antibodies on the immune response to IC51 vaccination in subjects from the pivotal immunogenicity trial has been published. Persons with pre-existing, vaccine-induced TBE antibodies had a significantly better immune response (GMTs at day 28) against JEV after receiving the first vaccination, indicating a partial crossreactivity

between these two flaviviruses. This effect disappeared after the second IC51 dose (82).

Data from the immunogenicity trials show that IC51 produces a good immunological response in adults with a 2-dose schedule of 6 µg at an interval of 28 days. This immune response lasts for at least 1 year after priming. There is good evidence that the PRNT used in all immunogenicity studies as a surrogate marker indeed measures seroprotection against JEV infection (71, 83).

Data on immunogenicity in children are currently not available from large phase III trials. A clinical phase II study investigating IC51 in children (1- < 3 years) showed favorable results concerning safety and immunogenicity (84-86). There is an urgent need for more pediatric data with respect to the potential use of the vaccine in endemic areas, where vaccination against JEV is given during childhood. Phase III trials establishing safety and immunogenicity in children are planned (70).

Open questions regarding the IC51 vaccine include the possibility of an accelerated vaccination schedule (e.g., days 0, 7 and 21) and trials investigating this are planned. Another area of interest with regards to immunogenicity are the boosting properties of IC51 in subjects who were primarily immunized with the currently available vaccine for travelers, JE-VAX®, or one of its licensed coproducts. Many subjects who received a basic immunization with JE-VAX® might be boosted with one dose of IC51, since there is only one serotype of JEV. Heterologous protection was demonstrated in preclinical trials. However, data regarding this issue are lacking and the use of different seed viruses with slight genetic differences might inhibit or at least weaken effects of crossprotection.

The application for a U.S. Biological License was submitted to the FDA in December 2007, as well as applications for European (EMEA, December 2007) and Australian (TGA, February 2008) marketing authorization. In the beginning of 2008, Intercell obtained the manufacturer's license for their production site in Scotland (84). IC51 was approved for adults by the TGA in February 2009, the FDA in March 2009 and by the EMEA in April 2009. Novartis is marketing and distributing IC51 under the trade name IXIARO® in Europe, North America and other selected markets. In Australia, the vaccine is licensed under the trade name JESPECT® and is distributed by CSL Biotherapies.

CONCLUSIONS

The risk of JEV infection is present in many regions of Asia and in the north of the Australian subcontinent (20, 31, 32, 34). Routine JEV immunizations have been shown to effectively decrease the rate of infections. Due to vaccine safety concerns, they were withdrawn in Japan but are still administered in China, South Korea and Vietnam. Other endemic countries, for example, Malaysia, Cambodia or Laos, have implemented specific immunization programs (16). Besides residents of endemic areas, travelers are a population at risk (87, 88). In the latter, the likelihood of infection with JEV is highly dependent on season, the circumstances of the journey (type and area of travel) and personal risk behavior (34, 47). In general, travelers appear to be at comparatively low risk for JEV infection (21), but cases are increasingly reported (34, 39, 42, 78, 89, 90). Visits to endemic rural regions with rice cultivation and breeding of domestic animals pose specific risks (27). Additionally, the risk of infection depends on the prevalence of mosquitoes and –at least in temperate climates– the season (15, 19).

According to the U.S. Advisory Committee on Immunization Practices, travelers should be vaccinated if they stay in endemic areas for at least 1 month or travel to rural areas (21). This recommendation was based on the safety profile of the formerly available mouse brain vaccine. An international group recommends counseling on JEV vaccination for all travelers to rural areas in Asia (38), and in particular for those with pre-existing medical conditions, with travel during transmission season and with plans for longer stays.

Recommending prophylactic immunization will be much easier in the future, while currently vaccination is restricted to travelers with an increased risk of acquiring JE. Persons at increased risk have been defined quite arbitrarily as travelers spending a month or longer in endemic areas, especially rural areas, during the transmission season (24). However, the possibility of an infection with JEV can never be ruled out when traveling to endemic areas, and several cases of severe JE have been reported in travelers after very short and apparently low-risk travel to endemic areas. Similar to the rare but fatal risk of a rabies infection, a JEV infection can well prove disastrous for the individual concerned. Therefore, this should be considered when counseling travelers who might be exposed to JE.

SOURCES

Intercell (DE); distributed in different markets by Novartis, CSL Biotherapies and Biological E.

DISCLOSURE

TJ participated as an investigator in several phase III trials of IC51. He received honoraria for presentations on the vaccine from Novartis Vaccines, the distributor of IC51 in Europe. AK is an employee of Intercell AG, the manufacturer of IC51.

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