



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

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ABSTRACT

Japanese encephalitis (JE) is the most common viral encephalitis in Asia. IC51 is a new Vero cell-derived, inactivated JE virus vaccine with non-inferior immunogenicity (after 2 months) compared to the US-licensed vaccine JE-VAX[®] (mouse brain-derived, inactivated) and with a more convenient (two injections instead of three) intramuscular dose schedule. Adult subjects from two studies were followed-up for comparative immunogenicity (JE-VAX[®]) at 6 months and long-term immunogenicity of IC51 alone at 12 months. At 6 months, immunogenicity was higher with IC51 (seroconversion rate [SCR] 95%; geometric mean titer [GMT] 84) than with JE-VAX[®] (SCR 74%; GMT 34). At 12 months, the SCR was 83% and the GMT (41) remained above the protective titer of 1:10. Most people immunized with IC51 will have protective neutralizing antibody levels for at least a year.

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1. Introduction

Japanese encephalitis (JE) is the most common viral encephalitis, with over 50,000 cases reported annually worldwide [1]. The JE virus is a mosquito-borne flavivirus and is endemic or epidemic in many tropical and subtropical countries in Asia. The majority of infections in humans are asymptomatic [2] but, when symptomatic, JE disease typically appears as a sudden onset of fever, complicated by neurological signs and symptoms due to neuroinvasion of the JE virus, and culminating in progressive coma. JE disease has an associated mortality rate of 30–40%, and 50% of surviving patients are left with permanent neuropsychiatric sequelae [3]. JE is a significant public health problem to persons who reside in endemic countries as well as to travelers. According to World Travel Organization figures, 18 million residents of Europe, United States (US) and Australia travel to JE endemic regions in South-East Asia annually and this figure is increasing [4]. There is no drug treatment for JE, only supportive care to ameliorate symptoms. Therefore, preven-

tive vaccination remains the single most important control measure worldwide.

Several first generation, inactivated JE vaccines have been produced by Asian national manufacturers for decades, mostly using mouse brains as a substrate for virus growth. More recently, Chinese manufacturers have produced inactivated and live virus vaccines, predominantly using a primary hamster cell substrate [5–7]. However, use has generally remained limited to domestic supply [8]. A formalin-inactivated, mouse brain-derived vaccine JE-VAX[®] is currently licensed in the US, Canada, Israel and Australia for the vaccination of travelers and military personnel. Although safety concerns had been raised in past years concerning the use of JE-VAX[®] [9–11], the Global Advisory Committee on Vaccine safety recently decided that the occurrence of a single case of acute disseminated encephalomyelitis reported after vaccination with JE-VAX[®] had no definite causal relationship to the vaccine, and thus, no definite evidence of an increased risk of acute disseminated encephalomyelitis following JE-VAX[®] administration can be drawn [12].

IC51 is a new purified, formalin-inactivated JE virus vaccine (virus strain SA₁₄-14-2) grown on Vero (African Green Monkey kidney) cells. In previous phase 2 and phase 3 studies, IC51 has

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demonstrated excellent immunogenicity at 2 months after vaccination, with between 95% and 100% of subjects being seroconverted [13,14]. A randomized, controlled phase 3 study showed IC51 to be non-inferior to JE-VAX® at 2 months after vaccination and with a favorable safety profile [14].

There is limited data on the duration of the immune response to JE virus vaccines. A trend for improved persistence of immunogenicity in IC51 recipients compared to JE-VAX® recipients was seen at 6 and 12 months after primary vaccination in a phase 2 study but the numbers of subjects were low [13]. The aim of this phase 3 follow-up study is to analyze the longevity of the IC51 immune response against JE at 6 and 12 months after primary immunization.

2. Methods

2.1. Subjects

The study population consisted of healthy male and female subjects, at least 18 years of age, who gave written informed consent. All subjects had completed one of two, randomized, controlled, blinded, 2-month, phase 3 trials (301 and 302) in which they had received at least one vaccination of either the investigational JE vaccine IC51, JE-VAX® or placebo. These clinical trials have been described previously [14,15].

For immunogenicity, of the 3258 subjects who had completed one of the prior, phase 3 studies according to the protocol, the first 298 eligible subjects (181 IC51, 82 JE-VAX® and 35 placebo) were enrolled and followed-up at 6 months (comparative immunogenicity population). These subjects had received primary immunization at four centers in Austria, Germany and Romania. In addition, the 181 subjects with IC51 were followed-up again at 12 months (long-term immunogenicity population).

For safety, the total population of the phase 3 studies of 3258 subjects (2283 IC51, 338 JE-VAX® and 637 placebo) were followed-up at 6 months for comparative safety while only the safety of 180 of the 181 IC51 12-month immunogenicity subjects were followed-up at 12 months for long-term safety (one subject was excluded due to a protocol violation: concomitant JEV vaccination).

The three treatment groups of the comparative immunogenicity population consisted of 181 IC51, 82 JE-VAX® and 35 placebo recipients and were comparable for all demographic and baseline characteristics. There were about equal proportions of men (43%, 47%, and 46% respectively) and women (57%, 53% and 54% respectively) and the mean ages were around 30 years of age (IC51 – 35 years, JE-VAX® – 32 years, and placebo – 28 years). Almost all subjects were Caucasians (96%, 100% and 97% respectively) (Fig. 1).

2.2. Vaccines

The investigational JE vaccine IC51 is manufactured by Intercell Biomedical Ltd., Livingston, UK. The attenuated SA₁₄-14-2 vaccine strain, adapted to primary canine kidney cells [16], was further passaged in Vero cells [13,17]. After purification, the vaccine is then inactivated with formalin using processes complying with current good manufacturing practices.

The finished product does not contain thimerosal, is adjuvanted with aluminum hydroxide and is filled into ready-to-use syringes. One dose of IC51 contains 6 mcg of purified virus adsorbed to 0.1% aluminum hydroxide. The vaccine was injected intramuscularly into the deltoid muscle on Days 0 and 28 of the previous active-controlled and placebo-controlled studies.

The comparator, JE-VAX® (BIKEN, Osaka, Japan), was injected subcutaneously into the upper arm on Days 0, 7 and 28 of the previous active-controlled study.

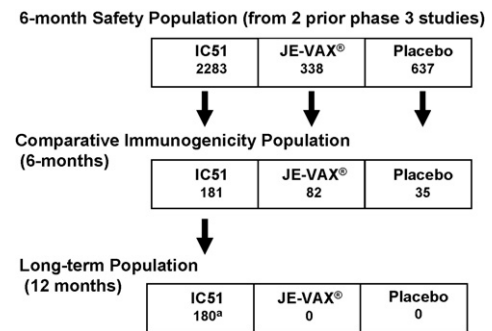


Fig. 1. Study populations: flow chart and subject numbers of 6-month safety population, comparative immunogenicity population and long-term population. IC51 recipients had completed either of the two phase 3 studies, JE-VAX® recipients had completed the pivotal immunogenicity study (301) and placebo recipients the pivotal safety study (302). Note: One subject was excluded in the long-term safety population due to a protocol violation: concomitant JEV vaccination.

Placebo was a 0.5 mL injection of 0.1% alum dilution, providing blinded injection schedules in both the active-controlled and placebo-controlled studies.

No investigational drug was administered in this follow-up study.

2.3. Study design

This was an uncontrolled, multi-center, phase 3, follow-up study designed to assess the immunogenicity of IC51 compared to JE-VAX® at 6 months and non-comparative, long-term immunogenicity at 12 months. Subjects were enrolled at 2 months after first vaccination in either of two prior phase 3 studies. Immunogenicity was assessed at 6 months in 298 subjects (181 IC51, 82 JE-VAX® and 35 placebo). The 12 month investigation was non-comparative and thus, immunogenicity was assessed only in the 181 IC51 subjects.

2.4. Immunogenicity assessments

Efficacy was measured by JE virus-specific neutralizing antibody titers, using the plaque reduction neutralization test (PRNT). This approach is standard for a disease such as JE where pre-existing protection in endemic countries makes a field trial impractical and the potentially fatal outcome makes a placebo-controlled trial unethical when an effective vaccine is available [18].

Virus neutralizing antibodies provide the best evidence that protective immunity has been established. A linear titer–protection relationship has been demonstrated, and efficacy studies in humans and animals corroborate the role of neutralizing antibodies in protection [9,18,19]. It is widely accepted that a JE virus neutralizing antibody threshold of 1:10 dilution producing a 50% reduction in plaque count in a PRNT (PRNT₅₀) is considered to be protective [19,20].

The PRNT determines anti-JEV neutralizing antibodies and does not measure non-neutralizing or cross-reactive antibodies. The JE virus SA₁₄-14-2 strain used in the PRNT will cross-neutralize other JE virus strains [21].

Serum samples were collected at 2, 6 and 12 months after first vaccination. Samples were stored at –80 °C and shipped on dry ice. The assay was performed in a central lab at Intercell AG, Vienna, Austria under good laboratory practice. PRNT was performed using a modification of the method described by Sukhavachana et al. [22]. Briefly, serial 4-fold dilutions of heat-inactivated serum (1:10; 1:40; 1:160; 1:640 or higher) were incubated for 1 h at 35 °C with a defined number of JE virus plaque forming units (400–800 pfu/mL)

and plated in triplicate onto monolayers of Vero cells with a methylcellulose overlay to restrict virus spread. After 5 days of incubation at 35 °C, the viral plaques were fixed, stained with paraformaldehyde/crystal violet, and automatically counted (ProtoCOL HR Colony Counter, Synbiosis, Cambridge, UK). PRNT50 titers (the serum dilution giving a 50% plaque reduction compared to plaque formation in virus-only controls) were calculated using a linear regression (probit) analysis program.

The seroconversion rate (SCR) was the percentage of subjects with a PRNT50 $\geq$ 1:10. The geometric mean titer (GMT) was calculated from PRNT50 values.

2.5. Safety assessments

Adverse events (AEs) were recorded at the 6 and 12-month face-to-face visits.

2.6. Statistics

The planned sample size was 160 IC51-treated subjects eligible for the immunogenicity population. A secondary objective was to investigate the comparative immunogenicity of IC51 and JE-VAX[®] in subjects at 6 months and long-term immunogenicity at 12 months after the first vaccination in two interim analyses (database cut-off dates 25 October 2006 and 12 March 2007). The 6-month analysis was performed on the comparative immunogenicity population and 12-month analysis on the long-term population.

Two-sided 95% confidence intervals (CIs) were calculated for the SCR according to the method recommended by Altman et al. [23]. The 95% CIs of GMTs were calculated by taking the anti-log of confidence limits estimated on the log-scale, assuming normality of the log-transformed PRNT50 values.

At 6 and 12 months, missing PRNT50 values from missing blood samples due to PRNT-negativity post-baseline or due to vaccination-related AEs were counted as PRNT-negative. All other missing PRNT50 values were imputed using a repeated measures analysis. A mixed model for repeated measures including a fixed time effect and assuming an unstructured within-subject covariance matrix was fitted to the given data. The missing values were estimated using the derived model.

A sensitivity analysis was performed on the percentage of seroconverted subjects at 12 months after the first vaccination by counting all missing PRNT50 values originating from missing blood samples as PRNT-negative (a worst case approach).

The SCR and the GMT were compared between IC51 and JE-VAX[®] subjects in the immunogenicity population at 2 months and 6 months after the first vaccination. Risk difference estimates and corresponding CIs were calculated using a Mantel–Haenszel type

estimator with stratification for center. The GMT ratio estimates and the corresponding CIs were calculated using an analysis of variance with center and treatment as factors.

3. Results

3.1. Demographics and disposition

The three treatment groups of the 6-month safety population ($N = 3258$) were similar for demographics and baseline characteristics. Mean age for the total population was 36 years (range 18–86 years) and there were slightly more women (56%) than men (44%). The majority of subjects (90%) were Caucasian.

In the immunogenicity populations, 172 IC51 (95%) and 61 JE-VAX[®] (74%) subjects had data for the 6-month, comparative assessments and 156 IC51 subjects (86%) for the 12-month assessment. Four subjects (2%) of the comparative immunogenicity population received prohibited flavivirus vaccinations during the study (tick-borne encephalitis, Yellow fever and JE); all four were seroconverted, with PRNT50 values within the overall distribution.

3.2. Immunogenicity

3.2.1. Comparative immunogenicity at 6 months

The SCR and the GMT at 6 months after first vaccination for IC51 and JE-VAX[®] are presented in Table 1 for the comparative immunogenicity population. At the start of the study (2 months after the first vaccination), the SCR was high for both IC51 (99%) and for JE-VAX[®] (98%) compared to placebo (14%). At 6 months after first vaccination, the SCR remained high in the IC51 group (95%) but had decreased markedly in the JE-VAX[®] group to 74% and placebo remained low (9%). The SCR risk difference estimate (IC51 minus JE-VAX[®]) was 17.8% with 95% confidence limits that excluded zero (6.75%, 28.86%), indicating a statistically significantly higher SCR for IC51 compared with JE-VAX[®].

The GMT in the IC51 group (311) was 3-fold higher than in the JE-VAX[®] group (100) at 2 months after first vaccination (placebo was 8.6). By 6 months, GMTs had decreased in both treatment groups but the GMT was twice as high in the IC51 group (84) than in the JE-VAX[®] group (34) and placebo was still low (6.2). The GMT ratio estimate (IC51/JE-VAX[®]) was 2.3% and the 95% confidence limits excluded zero (1.62, 3.17), thus showing the GMT in the IC51 group to be statistically significantly higher than in the JE-VAX[®] group. Based on observed values, only 5% of IC51 recipients had a 6-month PRNT50 below the protective titer of 1:10 compared with 27% of JE-VAX[®] recipients (Fig. 2).

Table 1
Immunogenicity up to 6 months after first vaccination for IC51 and JE-VAX[®]: comparative immunogenicity population

Time of assessment	IC51 (N = 181)	JE-VAX [®] (N = 82)	Treatment difference ^a
Seroconversion rate: subject number (%) [95% CI]			
2 months after first vaccination ^b	179 (99%) [96.1%, 99.7%]	80 (98%) [91.5%, 99.3%]	2.4% [–2.26%, 6.97%]
6 months after first vaccination ^b	172 (95%) [90.8%, 97.4%]	61 (74%) [64.0%, 82.6%]	17.8% [6.75%, 28.86%]
Geometric mean titer (min, max) [95% CI]			
2 months after first vaccination ^b	311 (5, 6435) [268.8, 359.4]	100 (5, 3438) [77.0, 128.7]	2.4 [1.79, 3.12]
6 months after first vaccination ^b	84 (5, 2218) [70.9, 98.4]	34 (5, 540) [25.1, 46.4]	2.3 [1.62, 3.17]

CI, confidence interval; Min, minimum; Max, maximum.

^a Treatment difference for seroconversion rate calculated as Mantel–Haenszel-type risk difference estimator (IC51 minus JE-VAX[®]) with 95% confidence interval, stratified for center. Treatment difference for geometric mean titer calculated as ratio (IC51/JE-VAX[®]) with 95% confidence interval from analysis of variance with factor treatment and center.

^b Missing values at 2 months have not been substituted; $N = 180$ for IC51 and $N = 82$ for JE-VAX[®]. Missing values at 6 months due to previous PRNT-negativity or due to vaccine-related adverse events were counted as PRNT-negative and PRNT50 was set to 5. All other missing values were imputed by estimation using a repeated measures model. For the SCR and GMT, there were 10 missing values for IC51 and 5 for JE-VAX[®].

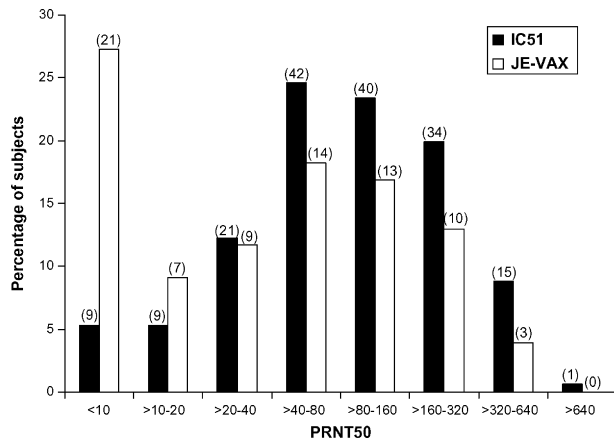


Fig. 2. Distribution of PRNT50 at 6 months in IC51 and JE-VAX® immunized subjects. Columns represent percentage of subjects; number of subjects is shown in brackets. Observed values only; missing values not imputed. Titers for PRNT50 = 50% reduction in plaque count with plaque reduction neutralization test.

3.2.2. Immunogenicity of IC51 at 12 months

Immunogenicity up to 12 months after first vaccination is presented in Table 2 for the long-term immunogenicity population. The SCR in IC51 recipients remained high (83%) after 12 months (95% CI: 77.3%, 88.1%) although it had decreased slightly over time. Based on observed values, only 18% of IC51 recipients had a 12 month PRNT50 below the protective titer of 1:10 (Fig. 3). PRNT50 values ranged from 5 to 538. A sensitivity analysis, in which all missing PRNT50 values were counted as PRNT-negative, gave a worst case lower bound for the SCR of 74% (95% CI: 67.2%, 79.9%) at 12 months. GMT decreased to 41 (95% CI: 34.4, 49.3) at 12 months after first vaccination.

3.3. Safety

The safety and tolerability of IC51 up to 2 months after vaccination has been reported previously [13,14]. The safety profile of IC51 during this long-term follow-up study (from 2 months after first vaccination) compared well with placebo and no safety concerns were identified. At 6 months ($N=3258$), the percentages of subjects with AEs (22% IC51, 18% JE-VAX®, and 24% placebo) and serious AEs (1.9% IC51, 1.5% JE-VAX®, and 2.4% placebo) were comparable for the three treatment groups. No AEs led to withdrawal from the study. The most common ($\geq 0.5\%$) AEs were also comparable for the three groups: nasopharyngitis (3.4% IC51, 4.7% JE-VAX®, and 4.9% placebo), influenza-like illness (1.1% IC51, 1.5% JE-VAX®, and 0.6% placebo), and headache (1.8% IC51, 0.3% JE-VAX®, and 1.1% placebo).

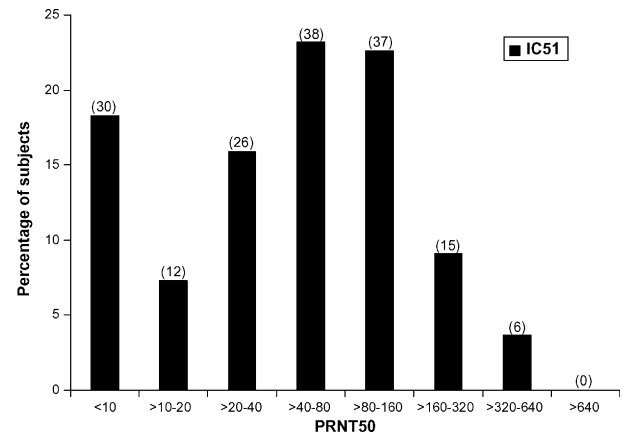


Fig. 3. Distribution of PRNT50 at 12 Months in IC51 immunized subjects. Columns represent percentage of subjects; number of subjects is shown in brackets. Observed values only; missing values not imputed. Titers for PRNT50 = 50% reduction in plaque count with plaque reduction neutralization test.

At 12 months ($N=180$) after first vaccination, the safety profile for IC51 was similar to that at 6 months, although the frequencies had increased due to the longer observation time: 34% had AEs and 2.8% had serious AEs. The most common ($\geq 2.0\%$) AEs were nasopharyngitis (3.9%), cystitis (2.8%), and headache (2.8%). There were no serious AEs that were considered as possibly or probably related to study medication and no AEs led to withdrawal from the study.

4. Discussion

The Vero cell-derived, inactivated JE virus vaccine IC51 has previously shown in phase 2 and 3 studies excellent immunogenicity up to 2 months after primary immunization and proven non-inferior to the US licensed JE-VAX® [13,14]. Results of a long-term, phase 2 study with IC51 suggested greater persistence of immunogenicity with IC51 compared to JE-VAX® over time [13], although the small numbers of subjects (7–17) made interpretation difficult. At 6 months, the immunogenicity of IC51 (SCR 100%; GMT 119) was higher than that of JE-VAX® (SCR 54%; GMT 50) and the difference was maintained at 12 months (IC51: SCR 100%, GMT 52; JE-VAX®: SCR 55%; GMT 30).

This phase 3 follow-up study has confirmed that the immune response following IC51 vaccination is more sustained than the immune response following JE-VAX® vaccination. In this study, after similarly high SCRs at 2 months, the SCR remained high in the IC51 group (95%) but had decreased in the JE-VAX® group (74%) at 6 months. The GMT in the IC51 group (84) at 6

Table 2
Immunogenicity up to 12 months after first vaccination for IC51: comparative and long-term immunogenicity population

	IC51 (N = 181)	95% confidence interval
Number (%) of seroconverted subjects		
2 months after first vaccination ^a	179 (99%)	[96.1%, 99.7%]
6 months after first vaccination ^b (comparative immunogenicity population)	172 (95%)	[90.8%, 97.4%]
12 months after first vaccination ^b (long-term immunogenicity population)	151 (83%)	[77.3%, 88.1%]
Geometric mean titer (range)		
2 months after first vaccination ^a	311 (5, 6435)	[268.8, 359.4]
6 months after first vaccination ^b (comparative immunogenicity population)	84 (5, 2218)	[70.9, 98.4]
12 months after first vaccination ^b (long-term immunogenicity population)	41 (5, 538)	[34.4, 49.3]

^a Missing values at 2 months have not been substituted; $N=180$.

^b Missing values at 6 months and 12 months due to previous PRNT-negativity or due to vaccine-related adverse events were counted as PRNT-negative and PRNT50 was set to 5. All other missing values were imputed by estimation using a repeated measures model. For the SCR and GMT, there were 10 missing values at 6 months and 17 at 12 months.

months was >2-fold higher than the GMT in the JE-VAX® group (34).

At 12 months, there was still a high level of immune response in the majority (83%) of IC51-treated subjects, with less than one-fifth (17%) of subjects with no detectable antibodies. The GMT (41) for IC51 remained above the protective titer of 1:10 at 12 months. Thus, this follow-up study confirmed in a larger subjects population the results of the phase 2 study [13].

The decline in GMT over time following IC51 vaccination seen in this follow-up study (a decrease to one-third of the initial GMT from 2 to 6 months and a decrease to one-seventh from 2 to 12 months) is expected after immunization with an inactivated vaccine and compares well with data from other inactivated JE vaccines. In a study in immunologically-naïve US soldiers, the GMT decreased down to one-third to one-fifth of the initial value at 6 months after primary immunization with JE-VAX® [24]. However, the soldiers were expatriated to endemic countries after immunization. In a study of individuals not exposed to natural flavivirus infections ($N=27$), GMT was 28 at 2 months after primary immunization with JE-VAX®; after 6 months, GMT declined to 4 [25]. The SCR dropped accordingly from 85% at 2 months to 33% by 6 months.

To our knowledge, there are no published data on the longevity of the neutralizing antibody response after vaccination with JE-VAX® in a non-endemic population. In US soldiers, 21 subjects who did not receive a booster vaccination retained elevated antibody titers for 2 years after primary vaccination (GMT 105) [20,21]. JE-VAX® is the only JE vaccine licensed outside Asia.

Overall, the safety profile of IC51 is excellent, and is comparable to placebo, which is critical for a vaccine for healthy recipients [15]. The AE and SAE frequencies were similar at 6 months for IC51, JE-VAX®, and placebo as were the frequencies for the most common AEs (<5%) of nasopharyngitis, influenza-like illness and headache. The safety profile for IC51 was consistent at 12 months.

Further studies are planned to investigate the optimum timing of a booster injection of IC51.

5. Conclusions

The majority of travelers immunized with IC51 will have Japanese encephalitis virus neutralizing antibody titers above the protective level of 1:10 for at least a year.

Conflict of interest statement

Four of the authors are employees of Intercell AG. All other institutions received funding from Intercell AG for contract research in conjunction with this clinical trial, from which salaries of the investigators have been partly paid.

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