European Medicines Agency

ASSESSMENT REPORT

FOR

IXIARO

Common Name: Japanese Encephalitis Vaccine (inactivated, adsorbed)

Procedure No. EMEA/H/C/000963

Assessment Report as adopted by the CHMP with
all information of a commercially confidential nature deleted.

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Intercell AG submitted on 07 December 2007 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for IXIARO, which was designated as an orphan medicinal product EU/3/05/348 on 24 January 2006. At the time of submission and validation, IXIARO was designated as an orphan medicinal product in the following indication: prevention of Japanese Encephalitis. At the time of orphan designation, the number of subjects at risk of this condition was estimated to be less than 3 per 10,000 persons in the EU.

The applicant applied for the following indication:
active immunization against Japanese encephalitis for adults. IXIARO should be considered for use in individuals at risk of exposure through travel or in the course of their occupation.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The application submitted is a complete dossier:
composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and bibliographic literature substituting/supporting certain tests or studies.

Scientific Advice / Protocol Assistance:

The applicant received Scientific Advice in May 2005 (EMEA/CHMP/SAWP/146087/2005) and Protocol Assistance in September 2007 (EMEA/CHMP/SAWP/414690/2007) from the CHMP. The Scientific Advice in 2005 pertained to quality, non-clinical and clinical aspects of the dossier and the Protocol Assistance in 2007 pertained to clinical aspects of the Paediatric Investigation Plan.

Licensing status:

The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP :

Rapporteur: **Christian K. Schneider** Co-Rapporteur: **Liv Mathiesen**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 7 December 2007.
- The procedure started on 30 January 2008.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 18 April 2008. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 18 April 2008. In accordance with Article 6(3) of Regulation (RC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- During the meeting on 27-30 May 2008, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 3 June 2008.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 22 August 2008.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 7 October 2008.
- During the CHMP meeting on 23 October 2008, the CHMP agreed on a List of Outstanding Issues to be addressed in writing by the applicant.
- The applicant provided responses to the CHMP List of Outstanding Issues on 17 November 2008.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 01 December 2008.
- During the meeting on 15-18 December 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to IXIARO on 18 December 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 15 December 2008.
- The Orphan criteria were reviewed at the March 2009 COMP meeting (3-4 March 2009). The COMP recommended the removal of the product from the Community registry of orphan medicinal products.
- The Applicant withdrew his Orphan Designation on 9 March 2009.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Japanese encephalitis (JE) is a mosquito-borne arboviral infection and the leading cause of viral encephalitis worldwide with an estimate of at least 50,000 cases of clinical disease per year. Children less than 10 years of age are primarily affected. JE is endemic in many Asian regions such as China, Korea, Japan, South-East Asia and India. In recent years sporadic epidemics have also been noted in previously less endemic areas such as Nepal, Sri-Lanka and Northern Australia. Residents of rural endemic regions are at highest risk and the disease occurs much less frequently in urban areas. The majority of infections remain asymptomatic and overt encephalitis occurs in only one out of 50 to 1,000 individuals infected.

In adults, JE disease typically follows an incubation period of 4-14 days and is mostly characterized by sudden onset of fever, chills, and aches, including headaches, mental confusion and sometimes nuchal rigidity. In children gastrointestinal pain and vomiting can be dominant initial symptoms and convulsions are very common. JE may present as a mild or moderate disease leading to an uneventful recovery or may rapidly progress to severe encephalitis with mental disturbances (e.g. confusion), general or focal neurological abnormalities (such as paralysis, movement disorders, abnormal posture, seizures) and coma. Out of the approximately annual 50,000 cases of JE more than 10,000 end fatally, and about 15,000 survivors are left with neurological and/or psychiatric sequelae requiring rehabilitation and continued care.

The overall risk of JE for travellers to endemic areas is considered rather low and was calculated to be about 1:5,000 to 1:20,000 per week. However, the risk may significantly increase when travelling in rural destinations and during the season of enhanced transmission (mostly May to September). The CDC reviewed cases of JE among expatriates and travellers that occurred during 1978–1992. From a total of 24 cases outcome information was available for 15 patients, of whom 6 died, 5 were listed as disabled, and 4 recovered. Only 2 of these 24 patients were tourists; the other patients were doing research or medical relief work or were soldiers. Further cases of JE were reported from tourists visiting Bali, Indonesia or Thailand.

The immune response to JEV infection has not been fully characterized, and both humoral and cellular responses may play a role. However, it is widely accepted that virus neutralizing antibodies provide the best evidence that protective immunity against JEV has been established. A linear titre-protection relationship has been demonstrated and data from efficacy studies in humans and animals corroborate the role of neutralizing antibodies in protection. Monoclonal antibodies to epitopes on the envelope

glycoprotein both neutralize JEV in vitro and protect mice from lethal challenge. Murine studies have also demonstrated that protection can be mediated through either adoptive transfer of T-lymphocytes or passive administration of antisera from mice infected with JEV. Historically, passive transfer of human post-infection sera to at-risk subjects was protective and correlated with detectable neutralizing antibodies in recipients.

Vaccination against JE remains the most important strategy to prevent against JE disease worldwide. There is currently no medication treatment available for JE and thus pre-exposure protection against the disease is essential. Several first-generation, inactivated JE vaccines have been produced by Japan, Korea, Vietnam and other national manufacturers for decades, mostly using mouse brains as a substrate for growth of the virus. More recently, Chinese manufacturers have produced inactivated and live virus vaccines, predominantly using a primary hamster cell substrate. However, the use of these vaccines has predominantly remained limited to domestic supply in these Asian countries.

The formalin-inactivated, mouse brain derived vaccine JE-VAX (virus strain Nakayama-NIH) is the only JE vaccine in routine use outside Asia and is currently licensed in the USA, Canada, Israel and Australia for the vaccination of travellers and military personnel against JE. Throughout the EU no vaccine against JE has been authorized and prophylaxis for travellers from the EU to JE endemic countries is commonly achieved by vaccination with JE-VAX. It is of note that the production of JE-VAX has been discontinued in 2007 and that exhaustion of existing stocks is therefore conceivable.

IXIARO is indicated for active immunization against Japanese encephalitis for adults. IXIARO should be considered for use in individuals at risk of exposure through travel or in the course of their occupation.
Two intramuscular immunisations of the vaccine given four weeks apart are intended to achieve optimal protection against JE. At current, no information on potential booster doses is available.

2.2 Quality aspects

Introduction

IXIARO is an inactivated whole virus vaccine for active immunisation against Japanese encephalitis (JE). The vaccine has been designed to elicit the generation of neutralizing antibodies that have been implicated in conferring protection against JE. Vaccine production is based on the neuro-attenuated JE virus strain SA_{14}-14-2 propagated in Vero cells. Virus harvests are purified, formalin inactivated and adsorbed to aluminium hydroxide. The vaccine is presented as a ready-to-use suspension for injection in a single-dose pre-filled syringe. Each dose of 0.5 ml (extractable volume set to ≥ 0.57 ml) contains JEV target total protein concentration of 6µg that corresponds to a potency of ≤ 460 ng $ED_{50,}$ adsorbed to 0.1% aluminium hydroxide. The vaccine is stored at 2°C-8°C and the manufacturer has proposed a preliminary shelf life period of 12 months.

Active Substance

The active substance of IXIARO is a sterile solution containing purified, inactivated Japanese Encephalitis whole virus, which has been propagated in Vero cells. The non adjuvanted active substance bulk solution is clear and colourless with a pH of 7.5.

- Manufacture

Manufacturing Process and Process Controls

The manufacturing process of the active substance bulk can be divided into the following steps:

- Virus propagation in Vero cells
- Filtering, pooling and concentration of the harvest
- Removal of Vero cell DNA
- Purification via sucrose gradient centrifugation

- Virus inactivation with formaldehyde
- Neutralisation of the formaldehyde with sodium metabisulphite and sterile filtration

A batch is defined by the volume of active substance obtained from the thawing of a specific number of vials of Vero Working Cell Bank, which are then expanded in cell culture and infected with the appropriate amount of Working Virus Seed Bank material. The batch size is specified.

Vero cells are used as substrate for the propagation of the attenuated Japanese encephalitis virus, strain SA_{14}-14-2. After virus inoculation and propagation, virus harvests are filtered, pooled, and concentrated. The concentrated virus pool is treated with protamine sulphate to remove Vero cell DNA. The virus pool is then purified by centrifugation through a sucrose gradient. The purified virus material, a pool of sucrose gradient fractions from the centrifugation is inactivated with formaldehyde, and then neutralized. Following neutralization, the inactivated virus is sterile filtered through two 0.2 µm filters. The active substance is processed immediately to Final Bulk Vaccine.

The following steps have been identified as critical and have been used for validation studies:

- Infection of Vero cells with Working Virus Seed Bank
- Reduction of host cell DNA
- Inactivation of virus by treatment with Formaldehyde
- Sterile filtration

The process development has been described from the developmental batches produced at the Walter Reed Army Institute of Research (WRAIR), USA to the commercial lots produced at the Intercell facilities and comparability reports have been provided. The equivalence of phase III and the commercial production processes lots have been demonstrated.

Cell substrate (cell banks)

The Vero cell line, used as substrate, was originally derived from the kidney of a normal adult African green monkey in Japan. The origin and the establishment of the current Master and Working Cell Banks are sufficiently documented.

JE virus (origin and seed virus)

Japanese encephalitis (JE) virus belongs to the genus Flavivirus and the family Flaviviridae. The genome of JE, like other flaviviruses, is a positive-polarity single-stranded RNA of about 11 kb in length, capped at the 5' terminus but lacking a poly (A) tract at its 3' end. JE particles are spherical enveloped viruses, about 50 nm in diameter.

The genomic RNA contains one long open reading frame encoding three structural proteins the envelope (E), capsid (C) and membrane (M, which is expressed as prM, precursor to M) proteins, at the 5' end and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) at the 3' end. The structural proteins are involved in the majority of the biological properties of the virus, such as cell surface receptor binding and cell entry as well as invoking immunological responses (Ni and Barrett 1995).

The wild-type parental virus, SA-14, was isolated from a pool of *Culex pipiens larvae* from Xian, China, following 11 passages in mouse brain. The derivation of the strain SA_{14}-14-2 was performed through an empirical process of serial passage performed in Beijing, China, in primary hamster kidney cells (PHK) and was given the strain designation SA_{14} clone 14-2 (also designated SA_{14}-14-2). No information is available concerning the quality of the animals, cell substrate, reagents or facilities involved in the attenuation of the SA_{14} strain in China. Following a risk calculation concerning the possibility of TSE contamination during the attenuation process done in China for the SA-14 virus strain the information provided by the Applicant was considered sufficient to justify the use of these materials.

At WRAIR, the SA_{14} clone 14-2 was first adapted to primary canine kidney (PDK) cells and then to Vero cells. The Master Virus Seed Bank was prepared at WRAIR under GMP conditions.
The Working Virus Seed Bank was established at Intercell Biomedical Ltd under GMP conditions.

Virus propagation in Vero cells

A pooled cell suspension is divided into the appropriate number of cell culture containers and incubated until virus is inoculated. Viral inoculation is done at a determined multiplicity of infection (MOI).
The virus harvests are filtered, pooled, and concentrated.

Purification and filling

The Vero Host cell nucleic acid from the concentrated harvest is removed and the batch is then further purified by centrifugation in a sucrose gradient. This purified material, is then inactivated with formaldehyde. Following neutralization of the formaldehyde, the inactivated virus is sterile filtered through two 0.2 µm filters. The filtered active substance is sampled for QC testing and then further processed to final bulk vaccine.

Control of Materials

The following starting materials of biological origin are used in the production of the preparation of JE vaccine: JEV, strain SA_{14}-14-2, Vero cell substrate, trypsin and foetal bovine serum.
Other raw materials and cell culture reagents have been used during the establishment of the MCB, WCB, MVSB, WVSB and during cell expansion, virus inoculation, harvest and concentration in the production process. In general, sufficiently detailed information has been provided on the source and control of the starting material of biological origin and other raw materials. Intercell's analytical methods used to test the raw materials include pH testing of the active substance, testing of osmolality using a calibrated osmometer, testing for the amount of endotoxin by kinetic chromogenic assay (Ph. Eur. 2.6.14 (Method D)) and determination of cell growth of the Vero cell line. The levels of residuals are also determined.

Control of Critical Steps and Intermediates

Suitable in-process controls are included throughout the cell culture, virus propagation, virus harvests and purification process steps.

Process validation and/or evaluation

Process validation and evaluation have been conducted for the upstream (virus propagation) and downstream processes, which include harvest filtration, pooling and concentration of the harvest, virus purification and inactivation and active substance sterile filtration.
In addition to an evaluation of the process consistency from the three consistency batches, specific studies have been undertaken with respect to validation of critical steps in order to demonstrate the suitability and the efficiency of these steps, which are (1) Infection of the Vero cells with Working Virus Seed Bank; (2) The reduction of host cell DNA; (3) The inactivation of virus by treatment with Formaldehyde (including plaque assay for detection of live virus) and (4) Active substance sterile filtration. to demonstrate the suitability and the efficiency of these steps. Some of these studies are still on going and are to be reported as follow up measures.

Manufacturing Process Development

The Japanese encephalitis (JE) whole virus vaccine has been developed at, and initially manufactured by Walter Reed Army Institute of Research (WRAIR), Forest Glen, MD, USA, (lots for Phase 1 and Phase 2 studies). Intercell sublicensed the rights to manufacture the vaccine from VaccGen International LLC, USA and transferred the process from WRAIR to Intercell's manufacturing facility in Livingston, Scotland. Phase 3 clinical materials were prepared in this facility. The manufacture of

the commercial active substance is also carried out in this facility, but in new clean rooms. Several important changes have been performed between the WRAIR production process and the Phase 3 and Commercial Process.

A Comparability Protocol was developed to evaluate the comparability of the manufacturing processes conducted at the WRAIR and Intercell facilities and the equivalence of vaccine manufactured for Phase 1 and 2 clinical trials and pivotal Phase 3 studies. Vaccine lots evaluated for the purposes of this protocol include the Phase 2 WRAIR lot (historical data only), small-scale lots manufactured at WRAIR and Intercell during process transfer, and five consecutive large-scale lots manufactured at Intercell, three of which were evaluated in Phase 3 studies. The commercial manufacturing process for the Active substance remains largely the same as the process used for manufacture of Phase 3 clinical trial material.

Comparability has been demonstrated for many of the parameters used to compare the WRAIR (Phase 2 & 3 material) with the Intercell (Phase 3 material) and comparability reports have been provided. Possible impurities are adequately controlled by in-process controls or at the level of active substance release testing.

Characterisation

The identity of the JE vaccine virus strain was performed by PCR sequence analysis, by SDS-PAGE , Peptide Mass Fingerprinting and morphology by electron microscopy.

In the literature, the molecular differences in the genomes of the virulent SA-14 and attenuated strain SA_{14}-14-2 (the precursor strain to the present JE vaccine virus strain, adapted to growth in primary dog kidney cells) were determined. Forty-five nucleotide differences, resulting in 15 amino acid substitutions, were detected by comparing sequences of the SA-14 and strain SA_{14}-14-2 genomes. PCR analysis of a specific region of the Japanese encephalitis virus was performed on the Master Virus Seed Bank (MVSB) and on the current Working Virus Seed Bank (WVSB).

Peptide Mass Fingerprinting was used both to confirm the identity of the 3 major bands seen on SDS-PAGE of the JEV proteome, and to detect and characterize distinctive tryptic peptides of the attenuated strain SA_{14}-14-2 JEV strain.

The Electron Micrograph structure of the JE virus from SA_{14}-14-2 Virus Seed banks and preparations of the active substance shows the spherical nature and that virus particles have dimensions between 40 and 60 nm in diameter.

Aggregation status of JEV samples, sucrose gradient purified (SGP) material and neutralised inactivated virus material (NIV), was further determined using Size Exclusion Chromatography with a Multi Angle Light Scattering (SEC-MALS) detection method.

Impurities

Impurities include substrate, virus and production process-related impurities.
Impurities derived from the manufacturing process, i.e. from the virus (JEV WVSB), cell substrate (host cell DNA, host cell protein, the cell culture media components, bovine serum) and residual from the production process (i.e. protamine sulphate, sucrose, formaldehyde, sodium metabisulphite) were examined by the Applicant. Process-related impurities also consist of potential contaminants (viral, mycoplasma, other microbial contaminants and TSE agents). The Applicant has supplied either adequate documentation or risk assessment for the reagents of animal origin used in production, when it comes to TSE agent contamination, concerning production in the United States or at Intercell in Europe. Information concerning the reagents and cell substrates used in China for attenuation of the wild virus is not available.
The Applicant also presented data on active substance-related impurities such as potential degradation products that could arise during manufacture and/or storage, including truncated viral forms, modified viral forms, non-inactivated virus and viral aggregates.

- Specification

The proposed testing protocol for the active substance bulk includes test methods for the appearance of the active substance solution, pH, sterility, protein content and JEV specific antigen content in addition to tests for residuals such as sucrose, formaldehyde, bovine serum albumin, host cell DNA and sodium metabisulphite. The Plaque assay is also included in order to detect potential residual infectious JE virus
The active substance content is controlled using the assay for total protein and a specific ELISA for JEV antigen. From both of these parameters, the specific activity in the active substance is calculated. These parameters have been implemented for active substance release testing.

As an interim antigen content-indicating parameter the measurement of the total protein concentration of the Active substance was performed. However, this method was not specific for the JE viral antigens. As requested, a quantitative ELISA for identification and quantification of JEV in the single virus harvest has been developed and validation data demonstrated the suitability of this assay. An ELISA method was also developed to determine the JEV specific antigen content in the inactivated active substance and formulation of vaccine batches to be produced from 2009 will be based on this parameter. It could be demonstrated that this ELISA method is also capable of demonstrating the identity of JEV in final vaccine lots in addition to being able to discriminate between JEV and flaviviruses from distantly related serocomplexes (i.e. Tick-borne Encephalitis Virus) and from closely related representatives from the same serocomplex (i.e. West Nile Virus).

Container Closure System

The Active substance is sterile filtered (2 x 0.2 µm filters) into a Stedim 50-L Flexboy bag before further processing. Information is provided on the manufacturer of the container closure system and its suitability with regards to mechanical, permeability, compatibility and extractables. Results from stability studies do not raise concerns regarding the container active substance compatibility.

- Stability

Since there are no specific stability data available to support the initial proposal for a 24 hours storage time at room temperature, formulation of the medicinal product will be done immediately after sterile filtration of active substance.

Medicinal Product

The vaccine is presented as a ready-to-use suspension for injection in a single-dose pre-filled syringe. Each 0.5 ml (extractable volume set to ≥ 0.57 ml) dose contains a target total protein amount of 6 µg corresponding to a potency of ≤ 460 ng ED_{50}. The composition of a final dose is depicted in the table below:

Composition of IXIARO per dose

Component	Quantity per dose (0.5-mL)	Function	Reference to standards
Active substance JE-PIV (Japanese Encephalitis - purified inactivated virus)	6.0 µg ± 1.2 µg (*) (Total protein) corresponding to a Potency ≤ 460 ng ED_{50}	Antigen	In-house specifications

Excipients			
Aluminium hydroxide, hydrated	0.1 % (vol/vol) (**)	Adsorbent/adjuvant	Ph. Eur.
Phosphate Buffered Saline (PBS) (***)	to 0.57 mL (****)	pH buffering agent/Active substance solvent	In-house specifications

(*) the total protein concentration is derived from the total protein concentration of the Active substance and the batch formula of the Final Bulk Vaccine, i.e. as 0.95 x total protein concentration of the Active substance.

(**) corresponding to approximately 0.25 mg Al^{3+}

(***) PBS 0.0067 M (PO_4)

(****) extractable volume

The vaccine does not contain any preservative or antibiotics. In addition to the components listed in the table, each dose of vaccine contains trace amounts of residual Vero cell protein, sucrose, formaldehyde, protamine sulphate and sodium metabisulphite.

- Pharmaceutical Development

IXIARO was initially developed and manufactured by WRAIR. Although the first GMP lot produced at WRAIR contained preservative (thiomersal), subsequent lots produced during product development and the commercial lots do not contain any preservative. Two clinical lots of IXIARO, filled in vials were produced at WRAIR for vaccinating human volunteers in Phase 1 and Phase 2 studies After transfer of the process from WRAIR to Intercell's manufacturing facility in Livingston, UK, Phase 3 clinical materials (Active substance and Final Bulk Vaccine) were prepared in the Livingston facility. Final filling into single dose syringes and packaging of the Phase 3 material was performed at Nova Laboratories (Wigston, Leicester, UK). The Final medicinal product bulk from the commercial scale production process is produced at Intercell Biomedical Limited, Livingston UK but filled into single dose syringes and packaged at Vetter Pharma-Fertigung GmbH & Co. KG, Ravensburg, Germany.

The Active substance is adsorbed onto 0.1% hydrated aluminium hydroxide. In initial formulation studies employing experimental vaccine batches, it was shown that adsorption of the purified inactivated JEV onto aluminium hydroxide occurred instantly with 88% of the antigen bound within 10 minutes and about 93% at 4 hours. Formulation of the commercial vaccine lots is in principle the same as formulation of the Phase 3 lots, which in turn is similar to the Phase 2 lots produced by WRAIR. In the course of product development, the adsorption process has been scaled up and the mixing procedure has been improved. The supplier of aluminium hydroxide has also been changed. Satisfactory information confirming the robustness and consistency of adsorption procedure chosen for the commercial product was gained by determining the adsorption rates of clinical consistency and commercial batches.

A Comparability Protocol to evaluate the comparability of the manufacturing processes conducted at the WRAIR and Intercell facilities and the equivalence of vaccine manufactured for Phase 2 clinical trials and pivotal Phase 3 studies has been provided. Intercell has initiated commercial scale manufacture of IXIARO Final medicinal product bulk in a new set of purpose-designed clean rooms entirely within the same manufacturing building at Livingston. This material is filled by a different Contract Manufacturer than was used for the Phase 3 clinical material. The filling is performed using an automated system at approximately 5-fold larger scale than was used for filling the clinical trial lots. The differences between the automated commercial filling process and the manual Phase 3 filling process have been outlined by the Applicant. Changes between Phase 3 manufacture and commercial

manufacture, including the change of aluminium hydroxide supplier and filling at a new contract manufacturer, have been assessed and are considered to be satisfactory.

- Adventitious Agents

Vero cells are used as substrate to propagate Japanese encephalitis (JE) virus using strain SA_{14}-14-2 Working Viral Seed produced at Intercell Biomedical Ltd., Scotland, UK.

The history of the establishment of the current JE Virus Seed Banks and current Vero Cell Banks are provided. Briefly, the virus strain SA14, isolated from a pool of Culex pipiens larvae following passages in mouse brain, was passaged in suckling mice and in primary hamster kidney (PHK) cells in China. Unfortunately, no information is available any longer on biological materials used during all these early passages in China.
The CHMP acknowledged that a live viral vaccine using the attenuated strain SA_{14}-14-2 is currently on the market in China and has been given to several million people. However, since the prevalence of BSE or TSE related disease is not known in China, it is not possible to determine if the live viral vaccine has been associated with any possible TSE related disease in the areas where it has been used.

During the procedure, the Applicant provided pertinent certificates and additional information regarding the TSE risk assessments of the virus seeds and cell banks employed for manufacturing, which was considered sufficient to justifying their use.

The Applicant has provided information on the cells used by WRAIR to adapt the virus strain SA_{14}-14-2 to grow in Vero cells and for the production of their master and working cell banks which have been used to produce the current working cell bank at Intercell. Information has also been given for the materials of biological origin used to produce both the virus seeds and the cell banks used.

Information on the biological materials used during the manufacturing process of IXIARO as reagents and media are presented and biological material derived for TSE-relevant animal species used during the manufacturing process are given. Information regarding compliance with TSE guidelines concerning packaging, filters and tubing from the manufacturing process is also provided.

Overall, sufficient information has been submitted to demonstrate compliance with the CHMP TSE NfG. The risk of transmitting TSE by IXIARO is considered very remote.

- Manufacture of the Product

The following sites are involved in the manufacturing: for IXIARO Intercell Biomedical Limited, Livingston, UK (formulation), Brenntag Nordic, Frederikssund, Denmark (manufacture of the aluminum hydroxide), Vetter Pharma-Fertigung GmbH & Co. KG, Schützenstrasse, Ravensburg, Germany (aseptic filling), Vetter Pharma-Fertigung GmbH & Co. KG , Holbeinstrasse, Ravensburg, Germany or alternatively Vetter Pharma-Fertigung GmbH & Co. KG , Mooswiesen, Ravensburg, Germany (labelling, packaging and storage). All manufacturing sites have been found to be in compliance with Good Manufacturing Practices (GMP) requirements.

Following adsorption of the active substance onto aluminium hydroxide, the final bulk is stored at Intercell Biomedical Limited until shipment to the filling plant Vetter Pharma-Fertigung GmbH & Co. KG, Germany. The final bulk vaccine is mixed continually during the filling process, and the product is dispensed into sterile single dose syringes which are automatically closed with sterile plungers by the filling machine.

The medicinal product is supplied as a sterile product and the formulation does not contain any preservative. A sterility test is carried out on the Final Bulk Vaccine and on the Final Vaccine Lot, as part of release testing.

The integrity of the container/closure system was verified and integrity was also determined during stability testing when sterility tests were performed on samples during and at the end of the stability study. To date, all results from sterility testing have met the specification confirming integrity of the container closure system.

- Product Specification

Release test methods and specifications for the Final Vaccine Lot include a test for identity, appearance, pH and extractable volume. Moreover the tests for sterility, bacterial endotoxins, pyrogenicity, general safety and free formaldehyde content are done according to Ph.Eur. A suitable Potency test is included as well as a test for residual aluminium and a test for degree of adsorption.

A method for JEV identity determination in the adsorbed medicinal product has been developed, where the JEV antigen is first desorbed from the aluminium hydroxide by incubation with a specific buffer and the released antigen is subsequently measured by a validated JEV ELISA. The Applicant has committed to conduct pyrogenicity testing until enough information is available to confirm that the bacterial endotoxin assay can be used as the release test. A test for residual formaldehyde on the final product has also been introduced to the medicinal product specifications.
To improve the potency determination for the medicinal product, a 2nd generation potency assay has been implemented by further development of the original 1st generation assay. The preliminary potency specification is based on the 2nd generation potency assay and has been set at "not more than 460 ng ED_{50}/dose".

- Stability of the Product

During the course of the licensing procedure the stability testing programme has been adapted to include not only appearance, pH, aluminium, potency, degree of adsorption, sterility and bacterial endotoxins but also specific activity determination. Satisfactory stability data from three manufacturing consistency batches stored at 5 ± 3° C are available for both the final bulk and the final vaccine lot. Supportive data are provided from stability of Phase 3 clinical batches. From the data submitted, the preliminary shelf life of 12 month period of validity for the final vaccine lot, and a 6 month period of validity for the final medicinal product bulk is considered acceptable. The preliminary shelf life assignment, however, has to be substantiated and to be regularly adjusted on the basis of upcoming results from the improved stability testing programme which includes potency determination using the 2nd generation potency assay. Corresponding commitments have been included in the table of follow-up measures.

Discussion on chemical, pharmaceutical and biological aspects

Information on the development, manufacture and control of the active substance and medicinal product has been presented in a satisfactory manner. Results from the tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance clinically.

At the time of CHMP opinion, there were a number of minor unresolved quality issues that had no impact on the risk benefit balance of the product. The Applicant provided a Letter of Undertaking in which he committed to resolve these issues as follow-up measures within an agreed timeframe after the opinion.

2.3 Non-clinical aspects

Introduction

The preclinical toxicity data package for IXIARO includes one formal GLP developmental and reproductive toxicity study performed and submitted. Pharmacological data submitted in support of IXIARO licensure are limited to primary pharmacodynamic studies. This reduced testing approach

was justified by the Applicant and agreed by the EMEA as part of a formal Scientific Advice procedure (EMEA/CHMP/ SAWP/146087/2005).

Pharmacology

- Primary pharmacodynamics

Pharmacodynamic studies performed with IXIARO include immunogenicity studies in mice, rats and rabbits; and also the active-, passive- and heterologous-protection studies against lethal challenge with JEV in mice. With the exception of a WRAIR's mice protection study using early batches (Lot #0574, #0475) of decreased potency, all pharmacological studies used vaccine lots manufactured using Intercell's process, as were used in the developmental toxicity study as well as the phase 3 clinical trials.

Detailed information on the studies and respective vaccine batches is summarized in Table 1.

Table 1: Overview of Pharmacology Studies

Type of Study	Species	IXIARO Lot Number	IXIARO Route + Dose + Test Criteria	Compliance Status
QC Tests performed for Batch Release				
Potency (PRNT assay*)	Mouse	Lot # 0475 Lot # 0574 Lot # 0737 ICB05/501 ICB05/502 ICB05/503 (All lots)	Two s.c injections on day 0 and day 28 0.2 mL of IXIARO serial dilutions Neutralizing Ab titres by PRNT on Day 42	GMP
Pharmacodynamic Studies				
Immunogenicity	Rat	D51029p1c	Four i.m. injections on days 0, 14, 28, and 42. 6 µg / 0.5mL IXIARO Neutralizing Ab titres by PRNT (day 21, 35, 49, 65)	N/A (GLP facility)
Immunogenicity	Rabbit	D51029p1c	Four i.m. injections on days 0, 14, 28, and 42. 6 µg / 0.5ml IXIARO Neutralizing Ab titres by PRNT (day 21, 35, 49, 65)	N/A (GLP facility)
(WRAIR) Active protection	Mouse	Lot # 0475 Lot # 0574	IXIARO serial dilutions on day 0 and day 14. Challenge with JEV SA14 (800 LD_{50} i.p). on day 28 Survival for further 21 days	N/A
(USAMC-AFRIMS) Active protection heterologous Protection	Mouse	ICB/05-501	Two i.p injections on day 0 and day 14. 170 µl of either IXIARO, JE-VAX or adjuvant Challenge on day 21 with JEV SA14 or JEV Beijing Neutralizing Ab titres by PRNT on day 21 (challenge day) Survival for further 21 days	N/A

In addition to the immunogenicity studies in mice using different batches of IXIARO as part of Quality Control Batch Release Test, an immunogenicity study was carried out in female rats and rabbits to evaluate whether these species were suitably responsive to IXIARO after 4 intramuscular injections given 2 weeks apart. Results showed that vaccination with IXIARO induced high levels of

JEV-specific neutralizing antibodies in all animals, with peak levels measured at day 35. There was no significant difference between rats and rabbits.

A challenge study (PN05-05/03) was conducted in mice to determine vaccine-induced active (heterologous)-protective efficacy against JE viruses. JE-VAX (BIKEN JE-VAX) was used as comparator.
The experiment was divided into 2 parts: Part 1 (PN05-05/03-1; homologous challenge with 1000 PFU SA14) and Part 2 (PN05-05/03-2; heterologous challenge with 500 PFU Beijing-1 strain: genotype III). Groups of 10 female ICR mice (6-7-week old) were immunized with 170µl of varying concentrations of the vaccine via intraperitoneal (IP) injection on day 0 and day 14. Approximately 7 days later (on day 21), mice were challenged intraperitoneally with 100µl of virus.
For SA14 challenge, the effective dose 50 (ED_{50}) for IXIARO was 4.7 ng (95% confidence interval: 2.6 – 8.3 ng) and ED_{50} for JE-VAX was 14.2 ng (95% confidence interval: 7.4 – 26.3ng). These results show that vaccination with both IXIARO and JE-VAX provide dose-dependent protection against lethal challenge with SA14.
Serum samples analyzed by $PRNT_{50}$ assay on challenging day demonstrated a dose-dependent relationship between vaccine doses and neutralizing antibody titre in both IXIARO and JE-VAX treatment groups. Furthermore, comparison of GMT titres and survival demonstrated a direct relationship between the antibody titre and survival of mice.
For mice challenged with Beijing-1 strain, only 20% control mice developed disease or died. Nonetheless, mice vaccinated with IXIARO or JE-VAX showed a statistically significant treatment trend, and immunization with higher dose of vaccine resulted in greater protection against heterologous challenge.

A passive transfer study with human immune sera (PN05-05/04) was conducted in mice to test protection and to correlate protection with *in vitro* $PRNT_{50}$ titres measured at Intercell.
Human immune sera were prepared from vaccinees enrolled in IC51-301 phase III trial, where subjects received either 2 immunizations (days 0 and 28) with IXIARO vaccine or 3 doses (days 0, 7 and 28) of JE-VAX. Sera collected on day 28 (IXIARO only) and day 56 post vaccination were pooled for actual antibody titres measured by the validated $PRNT_{50}$ test at Intercell.
For this passive transfer study, sera from IXIARO vaccinees were pooled into 4 batches: high titre (214), medium titre (43), low titre (21), and negative titre (inputed to 5). Similarly, sera from JE-VAX vaccinees were also pooled (55) as positive control. The experiment was divided into 2 parts: Part 1 (PN05-05/04-1: homologous challenge with 1000 PFU SA14) and Part 2 (PN05-05/04-2: heterologous challenge with 4000 PFU KE-093; genotype I).
Groups of 10 female ICR mice (6-7-week old) received human antisera in 0.5 ml via intraperitoneal injection and were then challenged intraperitoneally 17 to 18 hours later with a lethal dose of either SA14 or KE-093 strain. Challenged mice were observed for 20 days as described in Study PN05-05/03.
Blood samples were collected just before challenging and on day 21 for $PRNT_{50}$ antibody titres measured at Intercell.
A statistically significant treatment trend was detected for mice pretreated with JEV positive immune serum. The highest titered serum tested (107.5) rescued 9 of 10 mice upon challenge with the homologous strain SA14, with 60, 40, 30 and 0% survival observed in the next four treatment groups of input $PRNT_{50}$ titres 21.5, 21.4, 10.5 and 4.3 respectively. In the challenge experiment with the heterologous KE-093 strain, a statistically significant treatment trend was detected for mice pretreated with IXIARO antisera.

- Secondary pharmacodynamics

No secondary pharmacodynamics studies were performed with IXIARO.

- Safety pharmacology programme

No safety pharmacology studies were performed with IXIARO.
No cardiotoxic or respiratory specific risks were identified when IXIARO was administered as single or multiple doses in the immunogenicity studies in mice, rats and rabbits or in the rat pre- and post-natal development study. Thus the ommision of further safety pharmacology studies was considered justified.

- Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed with IXIARO.

Pharmacokinetics

Experimental studies to demonstrate absorption, distribution, metabolism, and excretion of the active ingredients in IXIARO have not been performed. This is in line with guideline CPMP/SWP/465/95.

Toxicology

The overall toxicology data package for IXIARO is limited to one formal toxicology study, namely, a pre- and post-natal toxicity study in rats, which was a pivotal GLP study. Animal tests performed during QC testing for batch release can give useful information on tolerability of IXIARO, however, they are not toxicity studies per se. An overview of toxicology studies performed with IXIARO is presented in Table 2.

Table 2: Overview of Toxicology Studies

Type of Study	**Species**	**IXIARO Lot No**	**IXIARO Route + Dose**	**Compliance Status**
Single dose - QC Tests performed for Batch Release				
General Safety Test (21CFR 610.11 and EP 2.6.9)	Mouse	All lots	Single i.p. injection 0.5 mL IXIARO	GMP
General Safety Test (21CFR 610.11 and EP 2.6.9)	Guinea Pig	All lots	Single i.p. injection 5.0 mL IXIARO	GMP
Toxicology Studies				
Pre- / post-natal developmental toxicity	Rat	ICB05/501	Two (day -7 before mating, day 6 after mating) or Three (day -21, day -7 before mating, day 6 after mating) i.m. injections. 6 µg/0.5mL of IXIARO Neutralizing Ab titers by $PRNT_{50}$ (day 21, 35, 49, 65)	GLP

- Single dose toxicity

No formal single dose toxicity studies were performed with IXIARO. However, single doses of IXIARO were tested in mice and guinea pigs in a general safety test performed as part of the routine Quality Control tests for batch release of the medicinal product, and no overt signs of ill health or weight loss was observed during the test period.

- Repeat dose toxicity (with toxicokinetics)

No formal repeat-dose toxicity studies were performed with IXIARO. However, the effects of multiple IXIARO doses were investigated in the primary pharmacodynamics (immunogenicity) studies in rats and rabbits, as well as in the pre- and post-natal development study performed in rats according to GLP. The study included histopathological evaluations.

- Genotoxicity

No genotoxicity studies were conducted which is in line with the Note for Guidance on Preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95)

- Carcinogenicity

No carcinogenicity studies were conducted which is in line with the Note for Guidance on Preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95)

- Reproduction Toxicity

A formal GLP-compliant reproductive and developmental toxicity was conducted in rats for the IXIARO vaccine. The suitability of rats as a model was demonstrated in previous preliminary immunogenicity study.
Groups of female rats (7 ~ 7.5 week old) received intramuscular injections of either IXIARO (Lot number: ICB05/501; 6 µg/0.5 ml) or placebo control (0.1% alum/PBS) with two vaccine schedules:
Vaccine I group and placebo control group: 3 injections at 14-day intervals, at 3 weeks (=day -21) and 1 week (=day -7) before mating, and on day 6 of gestation (= the start of organogenesis);
Vaccine II group: 2 injections at 14-day intervals, at 1 week (=day -7) before mating and on day 6 of gestation (= the start of organogenesis).
This design is based on the antibody profile of previous immunogenicity study and expected to coordinate the time of mating or gestation with low and high maternal $PRNT_{50}$ titres, thereby allowing to evaluate the effects of IXIARO vaccine on fertility and embryo-fetal development.

Based on examination of all adult females/each group no clinical observations, or haematology, clinical chemistry parameters and necropsy findings were noted that were attributed to treatment. On request, a histopathological examination was later performed on retained samples from this study, which encompassed all the major organs. The results indicated 4 females out of 20 that reared young to weaning, plus 4 that were not pregnant, in the vaccine II group (receiving 2 injections; one week before mating and on day 6 of gestation) had thymic atrophy, graded either minimal or focal minimal. The histological changes consisted of phagocytosis of cell debris in the cortex. Thymic atrophy was considered to be secondary to maternal stress. This finding was not observed in control or vaccine I group animals. There were no histology findings in other organs associated with administration of IXIARO.
With respects to foetal abnormalities, the only finding was an increase in the incidence of incomplete ossification in some parameters in the foetuses from females in the vaccine II group, as measured on Day 20 of gestation. These included incomplete ossification of >4 skull bones, pubes, ischia, sacral vertebral arches, and 2^{nd} and/or 4^{th} metacarpals. The incidence of the findings noted in vaccine II group (2 vaccinations) was higher than that in the concurrent control group and the vaccine I group (3 vaccinations) (which were also within the historical background range) and up to approximately 3 times greater than the highest value of the historical control ranges. Statistical significance was attained only for the following parameters: >4 skull bones and ischia ($p<0.05$ using the Fisher's Exact test). The increased incidence of incomplete ossification was generally due to an increase in the number of foetuses affected within the litter for pubis and sacral vertebral arches, but for the other parameters (including those which attained statistical significance) the number of litters affected was increased. In addition, incomplete ossification of skull bones is not an uncommon finding but to find >4 skull bones with incomplete ossification denotes an increase in severity and incidence of effect: incomplete ossification of >4 skull bones is usually not common. Examinations of the bone structure during the postnatal period were not performed.

- Local tolerance

No separate study was performed for local tolerance. The effects of multiple IXIARO doses given by intramuscular injection were investigated in the immunogenicity studies in rats and rabbits as well as in the pre- and post-natal development study performed in rats. No concerns were raised with respect to local tolerance.

- Other toxicity studies

Immunogenicity described under pharmacology. Other studies were not reported.

2.4 Clinical aspects

Introduction

Development and manufacture of the Japanese encephalitis whole virus, purified, inactivated vaccine (the ancestor of IXIARO) has been initiated at the Pilot Production Facility, Department of Biologics Research, of the Walter Reed Army Institute of Research (WRAIR) in Forest Glen, USA. Two small scale lots that have been employed for clinical phase 1 (lot 0574) and phase 2 (lot 0737) studies have been produced at the WRAIR. After sublicensing, the manufacturing process was transferred to Intercell´s facility in Livingston, UK, where clinical phase 3 material and commercial final bulks have been produced.

Clinical trial programme as conducted at the time of original dossier submission:

Clinical trials:
The clinical development program to support licensure of IXIARO consists of 9 studies in which approx. 4,700 subjects were enrolled and vaccinated. Two clinical studies are ongoing and interim results are available.

- Dose and regimen finding studies conducted at WRAIR, 49 subjects included
Studies WRAIR 763 and WRAIR815

- Pivotal studies providing the most relevant data to the formulation intended for commercialisation in a total of 3550 subjects enrolled.
IC51-301: To demonstrate non-inferiority of IXIARO against JE-VAX in terms of immunogenicity
IC51-302: To compare the safety and tolerability of IXIARO against placebo.
IC51-303: Follow-up to investigate persistence of immunogenicity up to 36 months

- Studies providing supporting immunogenicity data
IC51-309: To compare three different IXIARO batches
IC51-308: To address the effect of co-vaccination with Havrix 1440

- Additional safety data were available from the ongoing study
IC51-304: To compare standard two dose regimen to single dose regimen (only blinded day 56 safety data were available at time of initial submission)
IC51-305: long-term follow-up study to assess persistence of immunogenicity (only blinded 6-months safety data currently available)

During the procedure the Applicant has submitted results of clinical trial IC51-310 and clinical trial IC51-304. IC51-310 was a phase 3 study conducted to compare three commercial vaccine batches (consistency batches; IC51/07E/006A, IC51/07E/007A, IC51/07F/008A) in terms of efficacy and safety in 389 subjects (18 years of age and older).

GCP

The clinical trials were performed in accordance with GCP as claimed by the Applicant.

Pharmacokinetics

No clinical pharmacology studies describing the pharmacokinetic properties of IXIARO were conducted in support of this application, as the kinetic properties of vaccines do not provide useful information for establishing their pharmacological effects or adequate dosing recommendations. No information is available on the metabolism or excretion of IXIARO. Pharmacokinetic studies for aluminium hydroxide are considered dispensable since this is a well known adjuvant substance.

Pharmacodynamics

The pharmacodynamic principles of vaccines can be described as the induction of a qualitative and quantitative acceptable immune response within an acceptable time frame suitable to protect from infection with the wild-type antigen. Successful achievement of an anamnestic immune response is controlled by measuring surrogate parameters present in the serum of vaccinees like antibody concentrations (=antibody titres) or cell mediated immune responses. Such immunological markers above a specific threshold might serve as correlates for protection against JE. Pharmacodynamic studies assessing the neutralizing antibody response to JEV were assessed in Phase I, II and III studies.

The mechanism of action of the protective immunity conferred by IXIARO has not been fully characterized although it is known that protection is mediated by neutralizing antibodies. Apart from the assessment of immunogenicity (SCRs and GMTs using $PRNT_{50}$), no clinical studies have been conducted on the mechanism of action of IXIARO. Study WRAIR 763 assessed the presence of immunoglobulin (Ig) M and IgG using enzyme immunosorbent assays with limited success: 2 out of 25 subjects had IgG antibodies (1 subject who received 0.4 µg and 1 subject who received 2 µg IXIARO) and 1 out of 25 subjects had IgM antibodies (1 subject who received 2 µg IXIARO).

Although JE vaccine immunogenicity studies have focused on antibodies (and predominantly those directed against the E protein), cellular immune responses might also be important. It is possible that cellular immune responses alone might confer protection in seronegative vaccinees. Inactivated JE vaccines induce both JE-specific and flavivirus cross-reactive human lymphocyte antigen restricted T-cells. CD4+ and CD8+ T-cell memory has been demonstrated by lymphoproliferation in response to JE antigens. Data on cell mediated immune responses to vaccination with IXIARO are not contained in this submission but analysis of cellular immunity using peripheral blood mononuclear cells (PBMCs) will be performed for a subset of subjects enrolled in study IC51-301. Peripheral blood mononuclear cells have been isolated and cryo-preserved. Results will be forwarded to the agency upon availability.

The detailed characterization of the immunological response to IXIARO is discussed below.

Clinical efficacy

The analysis of clinical efficacy is performed on the generally accepted principle that a neutralizing antibody titre of ≥1:10 confers protection against JEV infection. This surrogate marker is widely recognised and used worldwide. In all clinical efficacy studies submitted for IXIARO a validated $PRNT_{50}$ assay was used to evaluate efficacy and the IXIARO vaccine strain SA-14-14-2 was used in the assay. A threshold of anti-JEV neutralizing antibody concentration of ≥1:10 was defined as surrogate marker for seroconversion.

An overview on studies performed to evaluate the efficacy of IXIARO is given in Table 3:

Table 3: Summary of efficacy studies

Study	Overview of Study Design	IXIARO Dose and Regimen	No Subjects Randomized Status
Pivotal Efficacy Study			
IC51-301	Randomized, active-controlled, observer-blind, non-inferiority (IXIARO vs. JE-VAX), phase 3 study	6 µg, Days 0 and 28	867 Completed
Supporting Studies			
WRAIR 763	Randomized, active (IXIARO)-controlled, single-blind, dose and schedule-finding, phase 1 study (IXIARO booster also given 8-9 months after 1st dose in subset)	0.4 µg, Days 0 and 28 0.4 µg, Days 0, 7 and 28 2 µg, Days 0 and 28 2 µg, Days 0, 7 and 28	25 Completed
WRAIR 815	Randomized, active-controlled (JE-VAX) open-label, dose and schedule-finding, phase 2 study	6 µg, Days 0 and 28 6 µg, Days 0, 14 and 28 12 µg, Days 0 and 28	94 Completed
IC51-303[1]	Multi-centre, follow-up, phase 3 study	Not applicable	6 months: 3258 12 months: 181 Ongoing
IC51-304	Randomized, active (IXIARO)-controlled, observer-blind, non-inferiority (1x 12 µg vs. 2x 6 µg), phase 3 study	6 µg, Days 0 and 28 6 µg, Day 0 12 µg (2x 6µg), Day 0	374 Completed
IC51-305[2]	Open-label, follow-up, phase 3 study (IXIARO booster given at month 11 and/or 23 if PRNT negative)	Not applicable	356 Ongoing
IC51-308	Randomized, active-controlled, single-blind, non-inferiority (IXIARO+HAVRIX vs. IXIARO, and IXIARO+HAVRIX vs. HAVRIX), phase 3 study	6 µg, Days 0 and 28	192 Completed
IC51-309[3]	Randomized, active (IXIARO)-controlled, double-blind, equivalence (of 3 clinical IC51 batches), phase 3 study	6 µg, Days 0 and 28	639 Completed
IC51-310	Randomized, active (IXIARO)-controlled, double-blind, equivalence (of 3 commercial IC51 batches), phase 3 study	6 µg, Days 0 and 28	389 Completed

1 Included subjects who had completed treatment in studies IC51-301 and IC51-302. Up to 12-month data available.
2 Included subjects who had completed treatment in study IC51-304 (6-month blinded safety data available).
3 In a follow-up study, a booster vaccination will be given to 200 subjects at Month 15 after the first vaccination in study IC51-309; follow-up will be for 12 months (study IC51-311).

- Dose response studies

Two clinical studies (WRAIR 763 and WRAIR 815) were performed to assess the dose-response relation. In addition alternative vaccination schedules were evaluated in study IC51-304.

WRAIR 763:

Healthy adults between the ages of 18 and 49 years were randomized equally to receive one of the following treatment arms:

- Group 1: 6 subjects, 0.4µg IXIARO (0.5 ml) i.m. injection on Day 0 and 28, and placebo (vaccine diluent) i.m. injection on Day 7
- Group 2: 5 subjects, 0.4µg IXIARO (0.5 ml) i.m. injection on Day 0, 7 and 28
- Group 3: 7 subjects, 2µg IXIARO (0.5 ml) i.m. injection on Day 0 and 28, and placebo i.m. injection on Day 7
- Group 4: 7 subjects, 2µg IXIARO (0.5 ml) i.m. injection on Day 0, 7 and 28

Blood samples were drawn at day 0 and 56.

Two subjects were not eligible for the per protocol population as they had received influenza vaccination (1 subject in group 1 and 1 subject in group 2). Thirteen of the above subjects received a single booster vaccination 8 to 9 months after the primary vaccination series.

Following the primary immunisation course only 29 – 71% of the subjects had seroconverted indicating an unsatisfactory immune response and an inappropriate dose range used.

None of the 13 returning subjects had detectable specific neutralizing antibody prior to their booster dose. Following booster injection, 12/13 (92%) subjects demonstrated seroconversion compared to 5/13 (38.5%) of these subjects at Day 56 following the primary vaccination series. There was an associated increase in GMT following booster injection compared with GMT at Day 56 following the primary vaccination series, with the exception of the highest dose group (cumulative dose 8.0µg).

WRAIR 815:

Subjects were randomized equally to receive one of the following treatment arms:

- Group 1: 6µg IXIARO (0.5 ml) i.m. injection on Days 0 and 28
- Group 2: 6µg IXIARO (0.5 ml) i.m. injection on Days 0, 14 and 28
- Group 3: 12µg IXIARO (1 ml) i.m. injection on Days 0 and 28
- Group 4: JE-VAX 1 ml subcutaneous injection on Days 0, 7 and 28

A follow-up visit was scheduled at Day 56, with optional visits at 6, 12, 18 and 24 months post dose 1 for assessments of immunogenicity. Blood samples were drawn for the determination of SCR and GMTs at days 0, 28, and 56 and for evaluation of antibody persistence at 6, 12, 18 and 24 months post dose 1.

Baseline characteristics showed that there were considerably more elderly women (9 male/15 female) and African Americans in group 2 as compared to the other three groups.

The primary endpoint was percentage of subjects with anti-JEV neutralizing antibody titres ≥1:10 (SCR) at Day 56. At Day 56, 74% of JE-VAX recipients had seroconverted, compared with 95-100% of subjects in the IXIARO groups. At Day 365, the seroconversion rate in each IXIARO group was 100%, compared with a SCR of 55% in the JE-VAX group. A similar trend was evident at Day 720, as the SCR was >83.33-100% in the IXIARO groups compared with 66.67% in the JE-VAX group.

At Day 56, GMT was highest in group 3 (two doses of 12µg IXIARO, 516.3) and lowest in group 4 (JE-VAX, 128.3). In all groups, GMT was higher at Day 56 than at subsequent time points, and was generally higher in the IXIARO groups than the JE-VAX group from Day 56 onwards.

Based on the results of these studies a regimen of 2 doses of 6µg was further evaluated in the pivotal efficacy study.

Study IC51-304:

Study IC51-304 was conducted with the aim of investigating alternative vaccination schedules, in particular whether or not single doses of either 6µg or 12µg of IXIARO would be equivalent to two doses of 6µg given 28 days apart.

The purpose of the primary immunogenicity analysis was to assess non-inferiority of IXIARO 1x 12µg vs. IXIARO 2x 6µg at Day 56 based on the difference in seroconversion rates (SCRs) in the per protocol (PP) population. Non-inferiority of 1x 12µg compared to 2x 6µg was accepted if the lower limit of the 95% CI of the adjusted SCR difference was higher than the non-inferiority margin at -10%. Results were expressed as the Confidence Interval (CI), the SCR difference and the Significance Level.

At Day 56 after the first vaccination, 28 of 66 subjects (42.4 %) from the Hamburg site and 19 of 48 subjects (39.6 %) from the Belfast site given 1x 12µg were seroconverted compared to 61 of 64 subjects (95.3 %) from the Hamburg site and 49 of 49 subjects (100 %) of the Belfast site given 2x 6µg. Adjusted SCR difference of 1x 12µg vs. 2x 6µg was -55.6 % [CI -65.3%; -45.9%] in the PP population. Results from IC51-304 clearly demonstrate that the 1x 12µg regimen is inferior to the 2x 6µg regimen proposed for licensure. Non-inferiority of the IXIARO 1x 12µg dose regimen to the

IXIARO 2x 6µg dose regimen was not demonstrated (p>0.99) in terms of seroconversion rates at Day 56 in the PP population (SCRs of 41.2 % vs. 97.3 %, respectively). Secondary immunogenicity analyses also included investigation of GMT values at all visits for anti-JEV neutralizing antibodies. The corresponding maximum values for GMT were 23 at day 28 for a single dose of 12µg and 266 for two injections of 6µg measured at day 35.
These data confirm that the dose-regimen of 2x 6µg 28 days apart is justified by the results of this trial.

- Main studies

Study IC51-301

Inclusion Criteria
Healthy male or female subjects who met all of the following criteria for inclusion in the study:
- At least 18 years of age.
- In female subjects either childbearing potential terminated by surgery or one year post-menopausal, or a negative serum pregnancy test during screening and the willingness not to become pregnant during the study period and 30 days after the last vaccination by practicing reliable methods of contraception
- Written informed consent obtained prior to study entry

Exclusion Criteria
Subjects who met any of the following exclusion criteria were not included in the study:
- Known history of hypersensitivity
- History of clinical manifestation of any flavivirus infection.
- History of vaccination against JE, Yellow fever and Dengue fever (an anti-JEV neutralizing antibody titre ≥1:10 at baseline was acceptable for inclusion, these subjects were part of the safety population and ITT population, but were not analyzed for immunogenicity in the Per Protocol [PP] analysis).
- Use of any other investigational or non-registered drug or vaccine in addition to the study vaccine during the study period or within 30 days preceding the first dose of study vaccine.
- Planned administration of another vaccine during the study period.
- Immunodeficiency including post-organ-transplantation or immunosuppressive therapy.
- A family history of congenital or hereditary immunodeficiency.
- History of autoimmune disease.
- Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs within six months of vaccination. (For corticosteroids, this included prednisone, or equivalent, ≥0.05 mg/kg/day. Topical and inhaled steroids were allowed).
- Any acute infections within four weeks prior to enrolment.
- History of severe hypersensitivity reactions (in particular to a component of the IXIARO vaccine, e.g., protamine sulphate), anaphylaxis or severe cases of atopy requiring emergency treatment or hospital admission.
- Infection with human immunodeficiency virus (HIV), hepatitis B or hepatitis C.
- History of urticaria after hymenoptera envenomation, drugs, physical or other provocations, or of idiopathic cause.
- Drug addiction within six months prior to enrolment (including alcohol dependence, i.e. more than approximately 60 g alcohol per day, or conditions which interfered with the study conduct).
- Inability or unwillingness to avoid more than the usual intake of alcohol during the 48 hours after vaccination.
- Diabetes mellitus in subjects receiving insulin therapy, severe cardiopulmonary disorders, history of malignancy in the past five years.
- Subjects with any condition which, in the opinion of the Investigator, made the subject unsuitable for inclusion.
- Pregnancy, lactation or unreliable contraception in female subjects

Treatments
Group A: 2 doses of IXIARO, 6 µg, i.m. on days 0 and 28 and one 0.5 ml injection of placebo (PBS containing 0.1% aluminium hydroxide) on day 7
Group B: 3 doses of JE-VAX (1.0 ml, s.c.) on days 0, 7 and 28.

Objectives
Primary Objective
To demonstrate the non-inferiority of IXIARO (2 x 6µg) compared to JE-VAX (3 x 1.0 ml) JE vaccine in terms of the SCR and geometric mean titre (GMT) at day 56; four weeks after the last vaccination.
Secondary Objectives
To compare:

- The superiority of IXIARO versus JE-VAX SCR and GMT at day 56, provided that non-inferiority had been demonstrated
- The immunogenicity of both vaccines in regards to SCR and GMTs of the North American with the European study population
- The immunogenicity of both vaccines in regards to SCR and GMTs in subjects older versus younger than 50 years of age
- The safety of both vaccines regarding changes in laboratory parameters and AEs including local reactogenicity

Immunogenicity endpoints
For immunogenicity assessment blood samples were drawn in all studies on day 0, 28 and 56.
The primary immunogenicity endpoint was the SCR (anti-JEV neutralizing antibody titre ≥1:10) and GMT (geometric mean of $PRNT_{50}$) at day 56 for the entire study population.

Laboratory methods
JE specific antibody responses were determined by a plaque reduction neutralisation assay as described below. Moreover in study IC51-301 a TBE ELISA was performed to determine baseline anti-flavivirus antibodies.

Plaque reduction neutralisation test ($PRNT_{50}$)
The $PRNT_{50}$ was performed in the Clinical Immunology Laboratory of Intercell AG, Vienna. Briefly, serial serum dilutions are incubated with a defined number of plaque forming units (400 pfu/ml) of JEV and plated in triplicate wells onto a monolayer of Vero cells. The neutralising antibody titre is expressed as the serum dilution giving a 50% plaque reduction ($PRNT_{50}$) compared to 100% plaque formation in virus only control. Seroconversion (the threshold antibody level for protection) was defined as a $PRNT_{50}$ titre ≥ 1:10, as recommended by the World Health Organization (WHO). All $PRNT_{50}$ results are given as reciprocal titres throughout the clinical documents. GMT was defined as the geometric mean of $PRNT_{50}$. Primary endpoints were assessed 4 weeks after the last vaccination in all studies, to ensure comparability of results. It should be noted that no international standard is available.
Assay validation was performed with regards of intra- and inter-assay precision, ruggedness, dilutional linearity and specificity. The specificity of the assay was evaluated by a JEV negative serum and sera from subjects vaccinated against Yellow fever virus or Tick-borne encephalitis virus, two closely related flaviviruses.

Other immunological assays

TBE-ELISA
A TBE enzyme-linked immunosorbent assay (ELISA) test, which does not discriminate between neutralizing and non-neutralizing anti-TBE antibodies and cross-reactive anti-flavivirus antibodies was performed at a certified laboratory for all serum samples from visit 0. Results from this test can reveal study subjects with pre-existing anti-flavivirus antibodies, mainly due to TBE vaccination, but potentially also to Yellow Fever vaccination or exposure to Dengue virus and West Nile Virus. Such pre-existing cross-reactive immunity may influence the kinetics of seroconversion to JEV as well as antibody titres.

Sample size
Anticipating the SCR of both vaccines being 75% and a non-inferiority limit of -10% for the SCR-difference "IXIARO minus JE-VAX", the Applicant calculated a total of 792 subjects (396 per group) necessary in order to demonstrate non-inferiority with a two-sided CI of 95% and a power of at least 80%
Assuming a SD of 0.4 (log10 units) for the IXIARO-GMT, a non-inferiority limit of 1/1.5 for the GMT ratio "IXIARO / JE-VAX.", a sample size of 109 subjects per group was calculated in order to demonstrate non-inferiority of the GMT with a two-sided 95%-CI and 80% power anticipating no true difference in GMTs between groups
Statistical methods
In general, data were described by means of statistical characteristics (categorical data: counts and percentages, continuous data: number of observations, mean, standard deviation (SD), minimum, median, and maximum) stratified for treatment group.

Immunogenicity analyses
Primary analyses for non-inferiority or equivalence for immunogenetic parameter were based on (pre-specified) per protocol (PP) populations while any assessment of superiority was based on the corresponding ITT population. The calculation of GMT ratios and their 95% CIs was performed by means of analyses of variance (ANOVA) with factors centre, age and treatment group applied to the log10-transformed $PRNT_{50}$ values. The anti-log of least square means group differences and their two-sided 95% CIs were calculated to achieve point estimates for the GMT ratios as well as the corresponding 95%-CIs.
Non-inferiority of IXIARO compared to JE-VAX in terms of the co-primary parameter SCR and GMT in study IC51-301 was assessed by a confidence interval approach:
A two-sided 95% CI for the SCR difference at day 56 between the IXIARO group and the JE-VAX group was calculated, using a Mantel-Haenszel type statistic stratified for centre and age group (<50 years versus ≥50 years). In case the lower limit of the confidence interval was > -10%, non-inferiority with respect to SCR was concluded. Non-inferiority with respect to GMT was concluded if the lower limit of the 95%-CI for the GMT ratio "IXIARO / JE-VAX" was above 1/1.5.
If non-inferiority had been shown with respect to SCR and GMT, superiority of IXIARO was assessed. Only if both, the lower limit 95%-CI for the SCR difference at day 56 between both groups was above 0 and the lower limit of the 95% CI for the ratio of GMTs at day 56 was above 1, superiority of IXIARO compared to JE-VAX was concluded.
In further analyses SCR and GMT on day 28 and day 56 were compared between treatment groups within the North American study population and, separately within each treatment group, between the North American and the European population. Explorative analyses were performed to assess the influence of age (<50 years versus ≥50 years), baseline anti-flavivirus immune status (in the IXIARO treatment group) as well as anti-JEV immune status at baseline (anti-JEV neutralizing antibody titre ≥1:10 vs <1:10) on immunogenicity parameter.

PP Population:
All randomized subjects without any protocol deviations. Subjects who were randomized incorrectly or took the wrong study medications were also excluded.
ITT Population:
All subjects randomized. Subjects were analyzed according to the treatment group to which they were randomized, rather than by the actual treatment they received.

Results
Recruitment
A total of 1271 subjects were enrolled in 11 study centres and 867 subjects from 10 study centres were randomized to treatment; 664 from North America and 203 from Europe. The first subject was enrolled on 05 September 2005 and the last subject completed on 17 March 2006.

Of the 867 subjects randomized to treatment, 430 were randomized to IXIARO and 437 to JE-VAX. 71 subjects (8.2%) did not complete the study and the reasons for discontinuation were withdrawn of consent (2.2%), adverse event (1.4%), protocol violation (1.2%) and lost to follow-up (1.2%)

132 subjects in the ITT population were excluded from the PP population due to major protocol deviations. The most common major protocol deviations were less than three vaccinations (29 [6.7%] subjects in the IXIARO group and 34 [7.8%] in the JE-VAX group), anti-JEV neutralizing antibody titre ≥1:10 at baseline (19 [4.4%] subjects in the IXIARO group and 18 [4.1%] subjects in the JE-VAX group), and no post-baseline seroconversion results (13 [3.0%] subjects in the IXIARO group and 15 [3.4%] subjects in the JE-VAX group).

Baseline data

In general the baseline characteristics of the study population were comparable for both vaccination groups. The median age was 41 years for both groups, with an age range of 18-79 years (IXIARO) and 18-80 years (JE-VAX), respectively. In both groups more female than male subjects were enrolled (IXIARO: 62.4%/37.6% vs JE-VAX: 59.3%/40.7%) and most study subjects were Caucasians (79% vs 82.5%) followed by Black (13.8% vs 14.4%).

Outcomes and estimation

Primary Analysis

The primary endpoints (SCR and GMT at Day 56) are presented in the Table 4 for the PP population, together with baseline results.

By definition of the PP population, no subjects were seroconverted at baseline. At Day 56, the proportion of subjects who had seroconverted was similar for both treatment groups (96.4% versus 93.8% for IXIARO and JE-VAX, respectively). The SCR risk difference estimate for IXIARO minus JE-VAX was 1.05% (95% CI: -1.33%, 3.43%). Since the lower 95% CI limit of the risk difference estimate (-1.33%) was >-10%, non-inferiority was demonstrated for Part I of the primary immunogenicity evaluation.

At Day 56, the GMT was over two times higher in the IXIARO group (243.6) than in the JE-VAX group (102.0). The GMT ratio for IXIARO/JE-VAX was 2.3 (95% CI: 1.97, 2.75). Since the lower 95% CI limit for the GMT ratio (1.97) was >1/1.5, non-inferiority was demonstrated for Part II of the primary immunogenicity evaluation.

Non-inferiority of IXIARO to JE-VAX was demonstrated for both components of the primary endpoint (both SCR and GMT at Day 56).

Table 4: SCR and GMTs: PP Population (Study IC51-301)

Time point	Treatment	N	SCR n (%)[1]	SCR risk difference[2] % [95% CI]	GMT[3] (SD/n)	GMT ratio[4] [95% CI]
Baseline	**IXIARO**	365	0 (0%)	NA	5.0[5] (0.0/365)	NA
	JE-VAX	370	0 (0%)		5.0[5] (0.0/370)	
Day 56	**IXIARO**	365	352 (96.4%)	1.05 [-1.33, 3.43]	243.6 (1163.1/361)	2.3 [1.97, 2.75]
	JE-VAX	370	347 (93.8%)		102.0 (221.0/364)	

Abbreviations: CI=confidence interval; GMT=geometric mean titre; n=number of subjects who seroconverted (for SCR) or with neutralizing antibody titres (for GMT); N= number of subjects in group; NA= not applicable; PRNT50=Serum dilution giving a 50% reduction of plaque counts in a plaque reduction neutralization test; SCR=seroconversion rate, SD=standard deviation.

[1] Percentages based on total number of subjects in the PP population for the respective treatment group

[2] Mantel-Haenszel type risk difference estimate (IXIARO minus JE-VAX) for seroconversion, stratified by centre and age group.

[3] For subjects with a minimum dilution factor <10 (PRNT50), the titre is set to 5

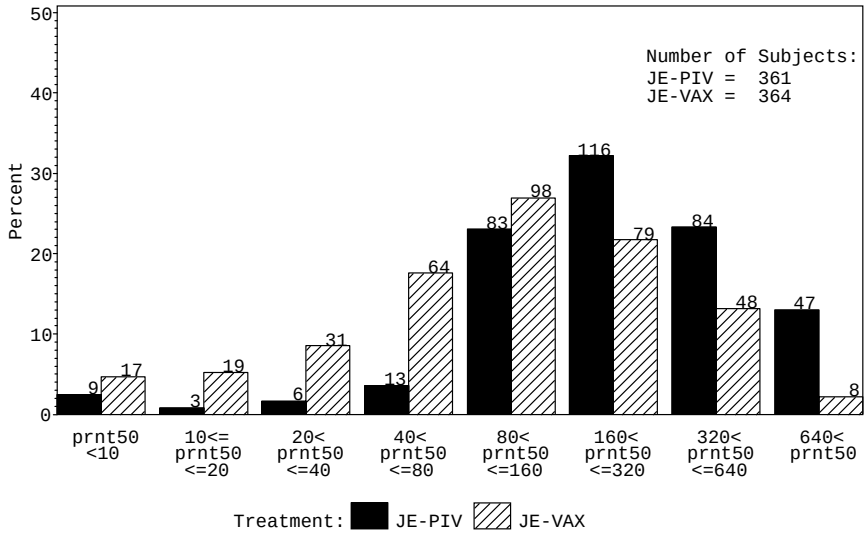
[4] Estimate for GMT ratio (IXIARO/JE-VAX) with 95% CI (from ANOVA with factors centre, age group and treatment).

[5] GMT of 5.0 was imputed for both groups at baseline.

In a post hoc analysis the results of the primary efficacy analysis were repeated in order to generate confidence intervals for the SCRs. At Day 56, the SCRs were calculated as 96.44% for the IXIARO group (95% CI: 94.00%, 97.91%) and 93.78% for the JE-VAX group (95% CI: 90.85%, 95.82%). At Day 56, the GMTs were calculated as 243.56 for the IXIARO group (95% CI: 216.44, 274.07) and 101.99 (95% CI: 90.28, 115.23) for the JE-VAX group.

The distribution of $PRNT_{50}$ values at Day 56 in the PP population is presented in Figure 1, demonstrating the greater proportion of subjects in the IXIARO (JE-PIV) group compared to the JE-VAX group with high $PRNT_{50}$ values. This is reflected in the higher standard deviation for the GMT in the IXIARO group (1163.1) compared to the JE-VAX group (221.0).

Figure 1: Distribution of PRNT50 at Day 56: PP Population



Secondary Analyses: SCR and GMT Analyzed by Age

The secondary analyses stratified by age revealed no significant differences in immunogenicity following the primary immunisation series between subjects ≤65 years of age compared to those >65 years of age (Table 5). In IXIARO recipients, SCR at Day 56 was 96.5% in subjects ≤65 years and 95.8% in subjects >65 years; GMT at Day 56 was 242.8 in subjects ≤65 years and 255.2 in subjects >65 years (Table 5).

Table 5: Seroconversion Rates and Geometric Mean Titres At Day 56 Stratified by Age ≤65 versus >65 years: PP Population (IC51-301)

Age (years)	SCR[1] n (%)		SCR risk difference[2] % [95% CI]	GMT		GMT ratio estimate[3] [95% CI]
	IXIARO	JE- VAX		IXIARO	JE-VAX	
≤65, N=692	329 (96.5)	330 (94.0)	2.10 [-0.68, 4.89]	242.8	102.3	2.37 [2.00, 2.81]
>65, N=43	23 (95.8)	17 (89.5)	11.11 [-4.12, 26.34]	255.2	96.8	2.54 [1.03, 6.28]
P-value	p=1.0000[4]	p=0.2201[4]		p=0.8389[5]	p=0.8440[5]	

1 SCR calculated from all values (missing values included in N).
2 Mantel-Haenszel type risk difference estimator (IXIARO minus JE-VAX) for seroconversion with 95% confidence interval, stratified for centre.
3 Estimate for GMT ratio (IXIARO/JE-VAX) with 95% confidence interval (from analysis of variance with factors centre and treatment).
4 P-value of Fisher's exact test for difference in seroconversion between age groups.
5 P-value of analysis of variance for difference in $PRNT_{50}$ between age groups.

Secondary Analyses: SCR and GMT at Day 56 analyzed by baseline anti-flavivirus status

SCRs and GMTs at Days 28 and 56 are summarized stratified by baseline anti-flavivirus status in Table 6 for the ITT population. There were no significant effects of baseline anti-flavivirus status on SCR or GMT in the IXIARO group at Day 56. However, positive baseline anti-flavivirus status did increase both SCR and GMT at the earlier Day 28 time point (SCR 76.5%; GMT 28.4) when compared to subjects with negative baseline anti-flavivirus status (SCR 49.0%; GMT 16.0).

Table 6: Seroconversion Rates and Geometric Mean Titers of Subjects Receiving IXIARO Stratified by Baseline Anti-Flavivirus Status[1]: Intent-to-Treat Population (Study IC51-301)

Time point	Immune status	N	SCR n/N (%)	GMT (n)[2]
Day 28	Positive	81	62/81* (76.5)	28.4* (81)
	Negative	339	166/339* (49.0)	16.0* (339)
Day 56	Positive	81	78/81 (96.3)	206.5 (81)
	Negative	339	310/339 (91.4)	186.7 (339)

*GMT=geometric mean titer; n=number of subjects who seroconverted (for SCR) or with neutralizing antibody titers (for GMT); N=number of subjects in group; NA=not applicable; PRNT50=Serum dilution giving a 50% reduction of plaque counts in a plaque reduction neutralization test; SCR=seroconversion rate. 1Determined by tick-borne encephalitis enzyme-linked immunosorbent assay. 2For subjects with a minimum dilution factor <10 (PRNT50), the titer is set to 5. *p<0.05 (based on Fisher's Exact test for difference between baseline anti-flavivirus status).*

History of TBE vaccination (primary immunization or booster vaccinations received within the previous 10 years) was recorded as present/absent at screening. In study IC51-301 prior TBE vaccination incidence was 12.4%.
Immunogenicity, based on SCRs and GMTs, was analyzed by history of prior TBE vaccination (Table 7). SCR at Day 28 (after 1st vaccination) was substantially higher in subjects with prior TBE immunization compared to those with no prior TBE immunization; 90.7% and 48.4%, respectively. Also GMT at Day 28 was higher in subjects with prior TBE immunization compared to those with no prior TBE immunization; 34.1 and 16.0 respectively. GMTs and SCRs were generally similar at Day 56 irrespective of whether subjects had a history of prior TBE vaccination.

Table 7: Seroconversion Rates and Geometric Mean Titers of Subjects Receiving IXIARO Stratified by Tick-borne Encephalitis Vaccination Status: Intent-to-Treat Population (IC51-301)

TBE status	SCR[1] n (%)	GMT (n)
Prior vaccination, N=108		
Day 28	49 (90.7)	34.1 (54)
Day 56	53 (98.1)	230.3 (54)
No vaccination, N=759		
Day 28	182 (48.4)	16.0 (376)
Day 56	344 (91.5)	182.7 (376)

GMT=geometric mean titer; n=number of subjects who seroconverted or with neutralizing antibody titers (for GMT); N=number of subjects in group; SCR=seroconversion rate; TBE=tick-borne encephalitis. [1] SCR calculated from all values (missing values included in N).

- **Supportive studies**

Long-term antibody persistence

Data on antibody persistence are currently available from study IC51-303. Further data are generated in on-going studies IC51-305 and IC51-311, in which antibody persistence and boosterability of the immune response are evaluated. Data from these studies are provided by the Applicant upon availability.

Study IC51-303 (Follow-up to studies IC51-301 and IC51-302)

Study IC51-303 is an ongoing, uncontrolled, multi-centre, phase 3 follow-up study to investigate the immunogenicity of IXIARO in subjects 24 months after the first vaccination. Healthy adults who were correctly enrolled in, and had completed IC51-301 or IC51-302 (pivotal safety study) with at least one vaccination were eligible for inclusion. The study started on 07 October 2005 in 44 centres (4 centres are involved in the immunogenicity analysis). The study has been amended to follow up subjects for 5 years.

Subjects were eligible for the long-term immunogenicity part of the IC51-303 study if they had been treated with IXIARO per protocol in studies IC51-301 or IC51-302 and had given informed consent for inclusion. The first 298 subjects reaching Day 1 of this study who were willing to participate in the immunogenicity part of the study were included. Following the database lock for studies IC51-301 and IC51-302, investigators were informed which subjects in the immunogenicity part had negative $PRNT_{50}$ values or had received JE-VAX or placebo; these subjects were discontinued.

Serum analysis for the presence of antibody to the JE virus was performed at 6 and 12 months (and will be performed at 24 and 36 months) using $PRNT_{50}$ assay.

The primary endpoint for the study is the SCR 24 months after the first vaccination. Secondary endpoints include SCR and GMT at 6, 12 and 36 months after first vaccination, and GMT at 24 and 36 months after vaccination. Immunogenicity results up to 12 months after first vaccination were presented.

The efficacy analysis is based on the ITT population: all subjects who were enrolled in the study, were planned to participate in the long-term immunogenicity part, and received IXIARO in a previous study. An additional sensitivity analysis was conducted, in which all missing $PRNT_{50}$ values were counted as PRNT negative.

At present interim reports on 6-months and 12 months long-term antibody persistence are available.

The results on persistence of antibodies up to 12 months are presented in Table 8. As expected and as usual for vaccines GMTs decrease over time and some individuals may drop below presumed seroprotection levels. Whether or not this is of any clinical significance can only be answered by boostering the study population. Such booster studies are ongoing but no data are yet available.

Table 8: 6- and 12-months long-term persistence of antibodies against Japanese encephalitis virus: $PRNT_{50}$, including GMT: ITT Population (Study IC51-303)

Time point	Descriptive statistics	IXIARO N=181
Month 2[1]	n	180
	GMT (95% CI)	310.8 (268.76, 359.44)
	SD	694.2
	Range	5 to 6435
Month 6[2]	n	181
	GMT (95% CI)	83.5 (70.89, 98.38)
	SD	196.9
	Range	5 to 2218
Month 12[2]	n	181
	GMT (95% CI)	41.2 (34.39, 49.33)
	SD	86.9
	Range	5 to 538

Abbreviations: CI=confidence interval; GMT=Geometric mean titre; N=number of subjects in group; n=number of subjects with data; SD=standard deviation

[1]Missing values at Month 2 have not been replaced.

[2] Missing PRNT50 values due to previous PRNT negativity or due to vaccine-related adverse events are counted as PRNT negative and PRNT50 is set to 5. All other missing values are imputed by estimation using a repeated measures model.

Heterologous neutralization potential

In addition to efficacy against the vaccine strain, relevance against other circulating JE-virus isolates becomes an important issue, in particular since IXIARO (JE-PIV or IC51) is a travellers' vaccine claimed to be effective against any of the worldwide circulating JE virus wild-types. The Applicant has presented relevant investigations in the original application and has conducted additional studies in order to support and strengthen validity of the first data set. All heterologous neutralization studies are summarized in the Table 9:

Table 9: Overview on in vitro heterologous neutralization studies

Data Set*	Clinical study	Sera	Analysis	Comment
2	IC51-301	88 JE-PIV 93 JE-VAX®	Winter 2005/2006	Randomly picked blinded set of study IC51-301; day 56 sera; PP population
3	IC51-301	22 JE-PIV 18 JE-VAX®	Summer 2006	Randomly picked blinded subset of Data Set 2
4	IC51-301	27 IC51 27 JE-VAX®	August 2007	PP population; d 56 sera; selected for a $PRNT_{50}$ GMT of ~240 (IC51) or ~100 (JE-VAX®) and a GMT ratio of 2.3; center ratio matched for US vs. Europe
5	IC51-303	27 IC51 27 JE-VAX®	December 2007/ January 2008	ITT3 population; m 6 sera; low GMT of 89 and 34 matched for $PRNT_{50}$ titer distribution and SCR
6	n/a	A; Nakayama-NIH killed vaccine B; negative control C; Duplicate of A D; P3 killed vaccine E; SA_{14}-14-2 life attenuated vaccine F; Beijing-1 killed vaccine G; Naturally Immune Donor H; Nakayama-NIH killed vaccine	January 2008	sera and data provided by R. Putnak, WRAIR and published by Ferguson et al.,2008
Pooled Data 1	IC51-301	94 sera (Data Set 3 and Data Set 4; 49 IC51 and 45 JE-VAX®)	Summer 2006 August 2007	
Pooled Data 2	IC51-301 IC51-303	103 sera (Parts of Data Set 2, Data Set 3, Parts of Data Set 4, and Data Set 5; 53 IC51 and 50 JE-VAX®)	Winter 2005 to January 2008	

The Applicant has presented a useful summary of the geographical distribution of different genotypes of JE virus and data on protection against heterologous JE virus. The strongest indication for heterologous protection is the fact that all vaccines against JE virus so far have been based on genotype III and they have proved to be efficient in different geographical regions harbouring different genotypic strains. However, classification according to genotyping is not necessarily well correlated to an immune response or vaccine efficacy. Therefore, the evidence of heterologous protection for IXIARO must mainly be based on submitted non-clinical and clinical data. Variations in antigenicity, neutralising antibodies, as well as protection (animal model), which have been reported among different isolates of JEV have to be taken into account. For example in the Japanese market the Nakayama strain was replaced by the Beijing strain on the reason that the Beijing strain gave a higher titres against homologous and heterologous JEV strains. The vaccine strain SA_{14}-14-2 itself has been passed >100 times in different cell cultures with loss of virulence and is thus at a distance from clinical isolates. A comparison with the use of live attenuated SA_{14}-14-2 vaccine used extensively in China is less relevant as a live vaccine induces a different immune response than an inactivated vaccine. Thus, even if cross-immunity is expected among different genotypes and strains of JEV, data has to be presented to ensure that the link to the documented effect of JE-VAX is strong.

In the initial application data for cross-immunity between SA_{14}-14-2 and the Nakayama strains were reported for a representative subset, Subset 2, of sera from the IC51-301 clinical study. The results are shown below:

Vaccine	*Assay virus*	*$PRNT_{50}$ GMT IC51-301*	*$PRNT_{50}$ GMT Subset 2*
IXIARO	SA_{14}-14-2	244 (n=361)	235 (n=88)
IXIARO	Nakayama		240 (n=88)
JE-VAX	SA_{14}-14-2	102 (n=364)	94 (n=93)
JE-VAX	Nakayama		1219 (n=89)

The neutralising titres in the sera from the IXIARO group were of the same size when measured against the SA_{14}-14-2 and the Nakayama strain. For sera from the JE-VAX group the titres against the homologous Nakayama strain was much higher than against the heterologous SA_{14}-1-4-2 strain.

Additional analysis of cross-immunity was performed on much smaller subset of sera, Subset 3, using 5 strains of JEV (all genotype III).

Study/Samples	**Vaccine**	**Assay virus**	**Seroconversion (pos/total)**	**$PRNT_{50}$ GMT**
Subset 3 22 IXIARO samples 18 JE- VAX samples	IXIARO	SA_{14}-14-2 Nakayama Beijing P- 20778 SA- 14	95% (21/22) 95% (21/22) 100% (22/22) 100% (22/22) 91% (20/22)	320 (n=22) 161 (n=22) 324 (n=22) 120 (n=22) 60 (n=22)
	JE- VAX	SA_{14}-14-2 Nakayama Beijing P- 20778 SA- 14	94% (17/18) 100% (18/18) 94% (17/18) 94% (17/18) 83% (15/18)	56 (n=18) 600 (n=18) 172 (n=18) 68 (n=18) 44 (n=18)

The Subset 3 was not representative for the whole set of sera (Subset 2). The neutralising antibody titre for the IXIARO group was 36% higher than in Subset 2, while for the JE-VAX group it was 50% lower than in Subset 2. Thus, a bias in favour of the IXIARO sera was introduced. While a direct comparison between IXIARO and JE-VAX is therefore not correct, it is demonstrated that both vaccines induce cross-immunity against a number of genotype III strains.

The Applicant has provided some new data on cross-immunity by adding more sera from clinical study IC51-301 to the analysis of neutralising antibodies performed with 4 JE virus strains (all genotype III). The sera were selected based on a mean value of GMT of 240 for IXIARO and a GMT of 100 for JE-VAX (measured against SA_{14}-14-2). The Subset 4 should therefore be representative for all samples from the trial.

Study/Samples	**Vaccine**	**Assay virus**	**Seroconversion (pos/total)**	**$PRNT_{50}$ GMT**
Subset 4 27 IXIARO samples 27 JE-VAX samples	IXIARO	SA_{14}-14-2 Beijing P-20778 SA-14	100% (27/27) 93% (25/27) 100% (27/27) 96% (26/27)	249 (n=27) 54 (n=27) 64 (n=27) 40 (n=27)
	JE-VAX	SA_{14}-14-2 Beijing P-20778 SA-14	100% (27/27) 100% (27/27) 100% (26/26) 100% (27/27)	109 (n=27) 100 (n=27) 82 (n=26) 53 (n=27)

Unfortunately the Nakayama strain (JE-VAX vaccine strain), was not included. In subset 4 JE-VAX performs slightly better than IXIARO, but good cross-immunity is shown for both.

In addition cross-immunity data has been presented based on 6 months sera from clinical study IC51-303. The sera were selected based on mean values of GMT of 89 for IXIARO and 34 for JE-VAX (measured earlier against SA_{14}-14-2).

Study/Samples	Vaccine	Assay virus	Seroconversion (pos/total)	$PRNT_{50}$ GMT
Subset 5 27 IXIARO samples 27 JE-VAX samples	IXIARO	SA_{14}-14-2	96% (26/27)	89
		Nakayama	85% (23/27)	33
		Beijing	85% (23/27)	49
		P-20778	85% (23/27)	29
		SA-14	81% (22/27)	33
	JE-VAX	SA_{14}-14-2	70% (19/27)	34
		Nakayama	96% (26/27)	206
		Beijing	67% (18/27)	22
		P-20778	63% (17/26)	18
		SA-14	80% (20/25)	26

In the Subset 5 sampled at 6 months after vaccination IXIARO performs slightly better than JE-VAX with respect to cross-immunity. In study IC51-303 half of the subjects were vaccinated with a batch of IXIARO which deviated from the other consistency batches in that it gave a much higher neutralising titre. This could have influenced the results at 6 months regarding cross-immunity. For example, the ratio of GMT between SA_{14}-14-2 and Nakayama for subjects vaccinated with IXIARO is close to 1 in the large Subset 2, while it is 2.7 in Subset 5.

In conclusion, the selection of subsets of sera used for analysis of cross-immunity has a large influence on the outcome. Nevertheless, even if there are differences, the data demonstrate that both IXIARO and JE-VAX are able to induce protective levels of neutralising antibodies against other JEV strains of genotype III. For IXIARO the link to the efficacy documented for JE-VAX will be the response of neutralising titre against the Nakayama strain (JE-VAX vaccine strain). While JE-VAX consistently induces much higher levels of neutralising titres against the Nakayama strain than IXIARO, the response in the IXIARO group is still high and considered protective.

The Applicant used a panel of JE virus isolates in order to document cross-neutralizing potential of IXIARO. All of these isolates are related however to genotype III. Since import of wild-type JE virus isolates belonging to other genotypes (I, II and IV) is subject to major restrictions the Applicant will establish necessary infrastructure in endemic regions in order to investigate cross-neutralizing activity of serum antibodies of individuals vaccinated with IXIARO against other, more recently collected isolates belonging to other genotype families.

Concomitant use studies

Study IC51-308 was a prospective, randomized, multi-centre, single-blind, active-controlled, phase 3 study to demonstrate the non-inferiority of IXIARO + Havrix as compared to IXIARO + placebo and Havrix + placebo in terms of immunogenicity. Havrix contains a sterile suspension of inactivated Hepatitis A virus adsorbed on aluminium.

The study was conducted at 3 centres in Germany and Austria between September 2005 and July 2006. Healthy adults aged ≥18 years were eligible for inclusion (subjects were excluded if they had a history of clinical manifestation of a flavivirus infection or HAV and/or if they had been vaccinated against JE, Yellow fever, Dengue fever or HAV). Subjects were randomized in a 1:1:1 ratio to receive one of the following treatment arms:

- IXIARO + Havrix: 6 µg IXIARO (0.5 ml, Lot ICB05-01 [Batch A]) i.m. on Days 0 and 28, and Havrix 1440 1.0 ml i.m. on Day 0
- IXIARO + placebo: 6 µg IXIARO (0.5 ml, Lot ICB05-01 [Batch A]) i.m. on Days 0 and 28, and placebo (0.5 ml PBS solution containing 0.1% aluminium hydroxide) i.m. on Day 0

- Havrix + placebo: Havrix 1440 1.0 ml i.m. on Day 0, and placebo (0.5 ml PBS solution containing 0.1% aluminium hydroxide) i.m. on Days 0 and 28

Blood tests for immunogenicity testing were taken at screening and Days 28 and 56. Serum analysis for the presence of neutralizing antibodies to the JE virus was performed using $PRNT_{50}$ assay. The immunologic assays regarding antibodies to HAV antigens (anti-HAV) were determined using the AxSYM HAVAB 2.0 Quantitative assay (Abbott Diagnostics). Due to low antibody titres obtained in the initial analysis a reanalysis of the sera was performed using the Enzygnost Anti-HAV competitive EIA.

The primary endpoints were GMT for anti-JEV neutralizing antibody at Day 56 and GMT for anti-HAV antibody at Day 28 (28 days after last active vaccination for IXIARO and Havrix, respectively). The primary immunogenicity analysis was conducted in the PP population (all randomized subjects without major protocol violations [possible violations included an anti-JEV neutralizing antibody titre ≥1:10 or anti-HAV antibody level ≥20 mIU/ml at baseline]).

The primary immunogenicity analysis was tested hierarchically. First, the IXIARO + Havrix group was tested for non-inferiority to the IXIARO + placebo group in terms of the GMT for anti-JEV neutralizing antibody at Day 56. If this test proved significant, a confirmatory test for non-inferiority of IXIARO + Havrix was performed, with the IXIARO + Havrix group vs. Havrix + placebo group in terms of the GMT for HAV antibody at Day 28. In both cases, non-inferiority was demonstrated if the lower bound of the two-sided 95% CI for the GMT ratio was >0.5. Though applying a hierarchical procedure, both tests were considered primary. If the first test failed, the second test was still applied, however in an exploratory manner.

GMT for anti-JEV neutralizing antibody at Day 56 and anti-HAV antibody at Day 28 (using the AxSYM HAVAB assay) are presented in Table 10 for the PP population.

Table 10: GMTs for Anti-JEV Neutralizing Antibody at Day 56 and Anti-HAV Antibody at Day 28: Per Protocol Population (Study IC51-308)

Parameter	n	GMT estimate (95% CI)	p-value*
Anti-JEV neutralizing antibodies, Day 56			
GMT[1]			
IXIARO + Havrix, N=58	58	202.7 (153.7, 261.2)	NA
IXIARO + Placebo, N=58	55	192.2 (147.9, 249.8)	NA
Ratios of GMTs			
IXIARO + Havrix/ IXIARO + Placebo[2]		1.0544 (0.7541, 1.4743)[3]	<0.0001
IXIARO + Havrix/IXIARO + Placebo[4]		1.1509 (0.7763, 1.7063)	<0.0001
Anti-HAV antibodies, Day 28			
GMT[1] (mIU/ml)			
IXIARO + Havrix, N=58	58	24.0 (19.1, 30.1)	NA
Havrix + Placebo, N=52	52	21.7 (17.2, 27.5)	NA
Ratios of GMTs			
IXIARO + Havrix/Havrix + Placebo[2]		1.1048 (0.8115, 1.5041)[3]	<0.0001
IXIARO + Havrix/Havrix + Placebo[4]		0.8809 (0.6314, 1.2292)	0.0005

Abbreviations: CI=confidence interval; GMT=Geometric mean titre, HAV: Hepatitis A virus; JEV: Japanese encephalitis virus; N=number of subjects in group; n=number of subjects with data; NA=not applicable. Observed values used.

** One-sided, non-inferiority.*

[1]GMT and CIs for individual treatment groups are calculated descriptively.

[2]Estimate for GMT ratios with CI (from analysis of variance with factors treatment and centre).

[3]Primary immunogenicity comparison.

[4]*Estimate for GMT ratios with CI (from analysis of variance with factors treatment and centre, including a treatment by centre interaction).*

Reanalysis of anti-HAV antibody titers using the Enzygnost Anti-HAV EIA resulted in higher anti-HAV antibody titers (GMTs of 124-150 mIU/mL) than those using the AxSYM HAVAB assay (GMTs of 22-24 mIU/mL) at Day 28. SCRs at Day 28 were 96%-100% using the Enzygnost EIA compared with SCRs of 65-74% using the AxSYM HAVAB. The GMTs and SCRs seen with the Enzygnost EIA are comparable to those reported from previous trials with Havrix.

Lot-to-lot consistency

Two lot-to-lot consistency studies were conducted.

Study IC51-309 was a prospective, randomized, multi-centre, double-blind, reference-controlled, phase 3 study to demonstrate the equivalence of 3 IXIARO batches in terms of immunogenicity. The study was conducted at 6 centres in Austria and Germany. The study started in September 2006 and the last subject completed in April 2007. Healthy adults aged ≥18 years were eligible for inclusion (subjects were excluded if they had a history of clinical manifestation of a flavivirus infection and/or if they had been vaccinated against JE, Yellow fever or Dengue fever).

Subjects were randomized in a 1:1:1 ratio to receive one of the following treatment arms:

- Batch A: 6 µg IXIARO (Lot ICB05-501) i.m. injection (0.5 ml) on Days 0 and 28
- Batch B: 6 µg IXIARO (Lot ICB05-502) i.m. injection (0.5 ml) on Days 0 and 28
- Batch D: 6 µg IXIARO (Lot ICB05-503) i.m. injection (0.5 ml) on Days 0 and 28

Blood tests for immunogenicity testing were taken on Days 0, 28 and 56. Serum analysis for the presence of neutralizing antibodies to the JE virus was performed using $PRNT_{50}$ assay.

The primary endpoint was GMT for anti-JEV neutralizing antibody at Day 56. Equivalence between batches of IXIARO was to be determined if all three pairwise 95% CIs for GMT ratios were between 0.5 and 2. The CIs of GMT ratios were calculated based on the observed PRNT50 values at Day 56 without imputation of missing values. An analysis of variance (ANOVA) was applied on the log-transformed $PRNT_{50}$ values including the factors centre and batch. The primary immunogenicity analysis was conducted in the PP population (all subjects in the ITT population without major protocol deviations [possible violations included a history of vaccination against JE, Yellow fever, or Dengue fever]). The ITT population comprised all randomized subjects, who had at least one dose of IXIARO, analyzed by treatment received.

As a follow-up study, it is planned to administer a booster vaccination at Month 15 to 200 subjects (who consent) who will then be followed up for a further 12 months.

As shown in Table 11 GMT at Day 56 was higher in Batch B (272.24) compared to Batch A (160.71) and Batch D (127.56). Only the GMT ratio for Batch A/Batch D had a 95% CI entirely covered by the predefined equivalence limits of 0.5 and 2, thus equivalence between the three batches was not met.

Table 11: Geometric Mean Titre at Day 56 and Pairwise Comparisons: PP Population (Study IC51-309)

Parameter	Treatment group (ratio)	GMT estimate	n	SD	$PRNT_{50}$ range	95% CI
GMT	Batch A, N=198	160.71[1]	197	304.2	5-2391	140.54, 183.76
	Batch B, N=202	272.24[1]	202	416.9	5-3017	237.22, 312.43
	Batch D, N=200	127.56[1]	200	209.7	5-1399	109.51, 148.57
GMT ratio	(Batch A/Batch B)	0.5857[2]	NA	NA	NA	0.4840, 0.7087
	(Batch A /Batch D)	1.2406[2]	NA	NA	NA	1.0249, 1.5018
	(Batch B/Batch D)	2.1183[2]	NA	NA	NA	1.7520, 2.5612

Abbreviations: CI=confidence interval; GMT=geometric mean titer; n=number of subjects with data; N=number of subjects in group; NA=not applicable; $PRNT_{50}$=Serum dilution giving a 50% reduction of plaque counts in a plaque reduction neutralization test; SD=standard deviation
Observed values used.
1 GMTs with CIs for single batches calculated descriptively. 2 Estimate for GMT ratios with CI (from analysis of variance with factors center and batch).

However, all three batches induced high seroconversion at Day 56. SCR estimates were 97.97%, 99.01% and 96.50% in Batches A, B and D, respectively (PP population). SCR estimates in the ITT population confirmed these results. Pairwise comparison of IXIARO batches demonstrated similarity of the three batches in terms of SCR.

Study IC51-310:
In order to demonstrate equivalence between clinical trial lots used in the pivotal clinical studies and the commercial process the Applicant has conducted a second lot-to-lot consistency study using the following 3 commercial lots produced at the Livingston, UK facility.

- **Batch A**: 6 µg IXIARO (Lot IC51/07E/006A) i.m. injection (0.5 ml) on Days 0 and 28
- **Batch B**: 6 µg IXIARO (Lot IC51/07E/007A) i.m. injection (0.5 ml) on Days 0 and 28
- **Batch C**: 6 µg IXIARO (Lot IC51/07F/008A) i.m. injection (0.5 ml) on Days 0 and 28

These lots were also well controlled for adsorption/desorption of the vaccine antigen from the AlOH adjuvant as well as for antigen/protein ratios which has not been the case for the clinical trial lots. Overall, the Applicant aims to demonstrating that potential inconsistencies in production identified for the clinical trials lots did not have much impact on clinical parameters, in particular efficacy as expressed by $PRNT_{50}$ titres.
Comparing results from the new lot-to-lot consistency study it becomes evident that firstly, individual lots do only differ minimally from each other as regards GMT estimates (see Table 12) and secondly, these results do not differ from those observed in previous studies.

Table 12: Geometric Mean Titre at Day 56 and Pairwise Comparisons: PP Population (Study IC51-310)

Parameter	Treatment group [ratio]	GMT estimate	n	SD	$PRNT_{50}$ range	95% CI
GMT	Batch A	160.80[1]	124	398.0	5-3589	133.51, 193.66[1]
	Batch B	188.21[1]	121	410.2	25-3645	163.77, 216.29[1]
	Batch C	168.43[1]	119	483.6	5-2992	136.20, 208.29[1]
GMT ratio	[Batch A/ Batch B]	0.8534[2]	NA	NA	NA	0.6630, 1.0984
	[Batch A/ Batch C]	0.9570[2]	NA	NA	NA	0.7426, 1.2333
	[Batch B/ Batch C]	1.1214[2]	NA	NA	NA	0.8689, 1.4474

Abbreviations: CI=confidence interval; GMT=geometric mean titre; n=number of subjects with data; NA=not applicable; $PRNT_{50}$=Serum dilution giving a 50% reduction of plaque counts in a plaque reduction neutralization test; SD=standard deviation. Observed values used.
1 GMTs and CIs for single batches calculated descriptively.
2 Estimate for GMT ratios with CI (from analysis of variance with factors centre and batch).

Seroconversion rates consistently exceed 95% for lots investigated in present (97.5%-100%) and previous studies. In front of this background potential variances in production of clinical trial lots are unlikely to have generated biasing clinical results and conclusions drawn from pivotal trials are consistent with those that can be drawn from trial IC51-310.

Further the impact of minor quality changes or potentially undetectable quality changes will be investigated in study IC51-314. In this study one batch used in study IC51-310 at 12, 18 and 24 months post filling will be evaluated. The Applicant is committed to provide interim and final CSR upon availability.

Clinical safety

- Patient exposure

Safety data are available from 9 clinical studies. These include one phase I study (WRAIR 763), one phase II study (WRAIR 815), and 7 phase III studies (IC51-301, IC51-302, IC51-303, IC51-304, IC51-305, IC51-308, and IC51-309). Overall, 3558 subjects who have received at least one IXIARO vaccination were compared to 435 subjects who received at least on JE-VAX vaccination, 65 subjects with one Havrix 1440 vaccination and 657 with a placebo (PBS solution containing 0.1% aluminium hydroxide) vaccination (Table 13). Study IC51-302 was the pivotal safety study including 2683 subjects. Data from all phase 3 studies except IC51-310 were included in a pooled 6-months safety analysis; the pooled analysis was performed before data were available from this study. Blinded data from studies IC51-304 and IC51-305 were included in the pooled 6-month safety analysis. Interim (6-months and 12-months) safety results from ongoing study IC51-303 and Day 56 safety results from study IC51-309 have been presented.

Table 13:

Study ID	Treatment	N	No of Dose	No. subjects (%)
Pivotal Safety Study IC51-302	IXIARO	1993	1 dose 2 doses	25 (1.3) 1968 (98.7)
	Placebo[1]	657	1 dose 2 doses	12 (1.8) 645 (98.2)
Pooled 6-Months Safety	IXIARO	3558	1 dose 2 doses	76 (2.1) 3482 (97.9)
	Placebo	657	1 dose 2 doses	12 (1.8) 645 (98.2)
	JE-VAX	435	1 dose 2 doses 3 doses	21 (4.8) 11 (2.5) 403 (92.6)
	HAVRIX	65	1 dose	65 (100)

- Adverse events

An adverse event (AE) was defined as any untoward medical occurrence in a subject administered an investigational product, whether or not related to treatment. Abnormalities already existing before the first administration of the investigational product were not considered as AEs, but were documented as medical history. All new abnormalities or any exacerbation in intensity or frequency (worsening) of a pre-existing condition during or after the first vaccination were documented as AEs.
Treatment-emergent adverse events (TEAEs) were defined as events which were not present at baseline, but started after the first vaccination or worsened with respect to severity following the start of treatment. AEs with missing start dates were considered treatment-emergent. Abnormalities already existing before the first administration of the investigational product were not considered as TEAEs, but were documented as medical history.

IC51-302 (pivotal safety study):
A total of 2683 subjects were randomized at 39 sites in Australia, Austria, Germany, United Kingdom (UK), Israel, New Zealand, Romania and the United States of America (USA). Informed consent was obtained from the subjects meeting all inclusion and no exclusion criteria prior to any study related procedures. After a screening period of up to 4 weeks subjects were randomized in a 3:1 ratio to one of the following two groups:

Verum Group: two injections of IXIARO (6 µg, intramuscularly [i.m.], 0.5 mL) on Day 0 and Day 28, or Control Group: two injections of placebo (PBS solution containing 0.1% aluminium hydroxide, 0.5 mL, i.m.) on Day 0 and Day 28.
A summary of TEAEs is provided in Table 14. During the total study period, 58.9% of subjects in the IXIARO (IC51) group and 56.6% of subjects in the placebo group experienced at least one TEAE.

Table 14: Overview of TEAEs (Total Study Period): Safety Population

Category	**IXIARO N=1993**		**Placebo N=657**			**Overall N=2650**	
Subjects with at least	**n**	**(%)**	**n**	**(%)**	**p-value**	**n**	**(%)**
one TEAE	1173	(58.9)	372	(56.6)	0.3159	1545	(58.3)
one severe TEAE	102	(5.1)	34	(5.2)	0.9192	136	(5.1)
one serious TEAE	10	(0.5)	6	(0.9)	0.2487	16	(0.6)
one possibly/probably related TEAE	774	(38.8)	254	(38.7)	0.9632	1028	(38.8)
one medically attended TEAE	254	(12.7)	80	(12.2)	0.7350	334	(12.6)
one TEAE leading to withdrawal	12	(0.6)	5	(0.8)	0.5857	17	(0.6)
Who died	0	(0.0)	0	(0.0)	-	0	(0.0)

N=number of subjects in group; n=number of subject with data; %=percentage of subjects based on number of patients in the group; TEAE=treatment emergent adverse even; p-value of Fisher's exact test for comparing treatment groups;

In total, 46.2% of subjects in the IXIARO group and 46.4% of subjects in the placebo group experienced TEAEs during the first vaccination period and the number of subjects experiencing TEAEs in the second vaccination period was lower (35.0% and 31.8% in the IXIARO and placebo groups, respectively).

The most common system organ class (SOC) for TEAEs were nervous system disorders (29.4% and 27.5% for IXIARO and placebo, respectively), general disorders and administration site conditions (22.3% for IXIARO and 23.0% for placebo), musculoskeletal and connective tissue disorders (18.0% for IXIARO and 18.3% for placebo), infections and infestations (13.8% for IXIARO and 13.2% for placebo) and gastrointestinal disorders (10.0% for IXIARO and 9.4% for placebo). There were no clinical relevant differences between the two groups.

The most common TEAEs reported in the total study period were headache (28.0% and 26.3% for IXIARO and placebo, respectively), myalgia (15.6% for IXIARO and 15.5% for placebo), influenza like illness (12.4% for IXIARO and 11.9% for placebo) and fatigue (11.4% for IXIARO and 11.7% for placebo).

For all systemic symptoms (headache, muscle pain, fever, flu-like-symptoms, nausea, vomiting and fatigue), the incidence one day after the first vaccination was slightly higher in the IXIARO group (the only exception being rash); after the second vaccination the rate of headache, fever and flu-like symptoms was slightly higher than in the placebo group. Local symptoms were most common on Day 0 decreasing over time for both treatment groups. The incidence of pain and tenderness was slightly higher in the IXIARO group compared to the control group (first vaccination, pain and tenderness: 18.5 % and 20.8 % in IXIARO group and 15.5 % and 17.4 % in the placebo group, respectively).

The severity of TEAEs was similar between the two groups (Table 15). In the safety population, 33.7% of subjects in the IXIARO (IC51) group and 34.1% of subjects in the placebo group had TEAEs that were mild in intensity. Corresponding frequencies for moderate TEAEs were 20.1% and 17.4%, respectively, and for severe TEAEs were 5.1% and 5.2%, respectively.

Table 15: Severity of Common TEAEs (Total Study Period) – Safety Population

TEAE system organ class[1] and preferred term[2]		IC51 N=1993		Placebo N=657		Overall N=2650	
		n	(%)	n	(%)	n	(%)
Any TEAE	**Severe**	**102**	**(5.1)**	**34**	**(5.2)**	**136**	**(5.1)**
	Moderate	**400**	**(20.1)**	**114**	**(17.4)**	**514**	**(19.4)**
	Mild	**671**	**(33.7)**	**224**	**(34.1)**	**895**	**(33.8)**
Gastrointestinal disorders	**Severe**	**18**	**(0.9)**	**4**	**(0.6)**	**22**	**(0.8)**
	Moderate	**54**	**(2.7)**	**17**	**(2.6)**	**71**	**(2.7)**
	Mild	**128**	**(6.4)**	**41**	**(6.2)**	**169**	**(6.4)**
Nausea	Severe	3	(0.2)	4	(0.6)	7	(0.3)
	Moderate	23	(1.2)	9	(1.4)	32	(1.2)
	Mild	105	(5.3)	36	(5.5)	141	(5.3)
General disorders and administration site conditions	**Severe**	**18**	**(0.9)**	**8**	**(1.2)**	**26**	**(1.0**
	Moderate	**121**	**(6.1)**	**41**	**(6.2)**	**162**	**(6.1)**
	Mild	**305**	**(15.3)**	**102**	**(15.5)**	**407**	**(15.4)**
Fatigue	Severe	8	(0.4)	5	(0.8)	13	(0.5)
	Moderate	59	(3.0)	22	(3.3)	81	(3.1)
	Mild	160	(8.0)	50	(7.6)	210	(7.9)
Influenza like illness	Severe	8	(0.4)	2	(0.3)	10	(0.4)
	Moderate	68	(3.4)	22	(3.3)	90	(3.4)
	Mild	172	(8.6)	54	(8.2)	226	(8.5)
Pyrexia	Severe	2	(0.1)	0	(0.0)	2	(0.1)
	Moderate	12	(0.6)	7	(1.1)	19	(0.7)
	Mild	50	(2.5)	13	(2.0)	63	(2.4)
Infections and infestations	**Severe**	**9**	**(0.5)**	**5**	**(0.8)**	**14**	**(0.5)**
	Moderate	**105**	**(5.3)**	**30**	**(4.6)**	**135**	**(5.1)**
	Mild	**162**	**(8.1)**	**52**	**(7.9)**	**214**	**(8.1)**
Nasopharyngitis	Severe	0	(0.0)	1	(0.2)	1	(0.0)
	Moderate	22	(1.1)	7	(1.1)	29	(1.1)
	Mild	72	(3.6)	18	(2.7)	90	(3.4)
Musculoskeletal and connective tissue disorders	**Severe**	**15**	**(0.8)**	**3**	**(0.5)**	**18**	**(0.7)**
	Moderate	**77**	**(3.9)**	**22**	**(3.3)**	**99**	**(3.7)**
	Mild	**267**	**(13.4)**	**95**	**(14.5)**	**362**	**(13.7)**
Myalgia	Severe	10	(0.5)	0	(0.0)	10	(0.4)
	Moderate	50	(2.5)	15	(2.3)	65	(2.5)
	Mild	251	(12.6)	87	(13.2)	338	(12.8)

Continued

TEAE system organ class[1] and preferred term[2]		IC51 N=1993		Placebo N=657		Overall N=2650	
		n	(%)	n	(%)	n	(%)
Nervous system disorders	**Severe**	**37**	**(1.9)**	**14**	**(2.1)**	**51**	**(1.9)**
	Moderate	**144**	**(7.2)**	**52**	**(7.9)**	**196**	**(7.4)**
	Mild	**404**	**(20.3)**	**115**	**(17.5)**	**519**	**(19.6)**
Headache	Severe	29	(1.5)	11	(1.7)	40	(1.5)
	Moderate	131	(6.6)	50	(7.6)	181	(6.8)
	Mild	399	(20.0)	112	(17.0)	511	(19.3)

Source: Section 14, Table 4.2.4.1; N=number of subjects in group; n=number of subject with data; %=percentage of subjects based on number of patients in the group; TEAE=treatment emergent adverse event; 1 Only includes SOCs in which TEAEs were reported in ≥2% subjects overall 2 Preferred terms only given for TEAEs occurring in ≥1.5% subjects overall

The pattern of causality was similar between the two groups. There were no clinical relevant differences in causality between the two groups. In the safety population, 38.8% subjects in the IXIARO group and 38.7% subjects in the placebo group experienced TEAEs that were possibly or probably related to study treatment (or missing causality).

The most common SOC for treatment-related TEAEs was nervous system disorders (22.0% and 20.4% of subjects for IXIARO and placebo, respectively), followed by general disorders and administration site conditions (17.2% for IXIARO and 18.1% for placebo), and musculoskeletal and connective tissue disorders (13.8% for IXIARO and 15.4% for placebo).

The most common treatment-related TEAEs were headache (21.5% and 19.9% of subjects for IXIARO and placebo, respectively), myalgia (13.6% for IXIARO and 14.3% for placebo), fatigue (9.4% for IXIARO and 9.9% for placebo) and influenza like illness (8.9% for IXIARO and 8.7% for placebo). A higher proportion of subjects experienced treatment-related TEAEs during the first vaccination period (31.6% and 33.0% of subjects for IXIARO and placebo, respectively) compared to the second vaccination period (20.0% for IXIARO and 18.1% for placebo).
The severity of treatment related TEAEs was similar between the two groups.
Very few medically attended TEAEs were considered related (2.1% of subjects in the IXIARO group and 2.0% of subjects in the placebo group).

Pooled 6-Months-Analyses

6-Months Pooled Safety Population
Of the 4715 subjects who were included in the 6-months pooled safety population, 1.5% completed the 6-months safety visit. Thereof 3558 received at least one IXIARO vaccination, 435 subjects received JE-VAX, 65 received Havrix, and 657 received placebo. The mean age was 35.2 years (range: 18 to 86 years) and 82.7% of subjects were aged between 18 and 49 years, 13.9% between 50 and 64 years, and 3.4% were 65 years or older. Subjects tended to be older in the JE-VAX group (mean 41.1 years) and younger in the Havrix group (mean 28.1 years). There were slightly more females (56.0%) than males (44.0%).

The objective of this analysis is to provide a compiled overview of the safety and tolerability of IXIARO as observed in the ongoing clinical trial program supplemental to the specific clinical study reports. The comparator groups (JE-VAX/Havrix1440/placebo) of these clinical trials provide information on background incidences and are used to validate the observed safety profile. The definition of the safety analysis population was identical in all clinical trials covered.

An overview on TEAEs is presented in Table 16.

Table 16: Treatment-Emergent Adverse Events (TEAEs)

Category	IXIARO N=3558		JE-VAX N=435		HAVRIX N=65		Placebo N=657	
Subjects with at least	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
one TEAE	2282	(64.1)	279	(64.1)	31	(47.7)	402	(61.2)
one severe TEAE	207	(5.8)	19	(4.4)	3	(4.6)	42	(6.4)
one serious TEAE	38	(1.1)	3	(0.7)	0	(0.0)	13	(2.0)
one related TEAE[1]	1362	(38.3)	149	(34.3)	12	(18.5)	255	(38.8)
one medically attended TEAE[2]	668	(19.4)	36	(10.7)	11	(16.9)	129	(19.6)
one TEAE leading to withdrawal	27	(0.8)	8	(1.8)	0	(0.0)	5	(0.8)
Who died	1	(<0.1)	0	(0.0)	0	(0.0)	0	(0.0)

1) Events with a causality reported as probable or possible or with a missing classification were considered related to study medication.
2) Medically attended TEAEs were not collected during study IC51-304 and IC51-301(in which JE-VAX was administered) but were collected during follow-on study IC51-303. Therefore the JE-VAX column represents medically attended TEAEs collected from Month 2 to Month 6. All subjects in the pooled 6-month safety population receiving JE-VAX originate from study IC51-301.
Abbreviations: N=number of subjects in group; n=number of subjects with event; %=percentage of subjects based on number of subjects in the group; TEAE=treatment-emergent adverse event.

TEAEs were experienced by similar percentages of subjects in the IXIARO group (64.1%), JE-VAX group (64.1%) and placebo group (61.2%), and by fewer subjects in the HAVRIX group (47.7%).
The incidence of TEAEs with IXIARO was similar to placebo for severe TEAEs (5.8% and 6.4% of subjects, respectively), SAEs (1.1% and 2.0%, respectively), treatment-related TEAEs (38.3% and 38.8%, respectively), medically attended TEAEs (19.4% and 19.6%, respectively), and TEAEs leading to withdrawal (0.8% for both groups).
Although the incidence of medically attended TEAEs was similar in the IXIARO group (19.4%) and the placebo group (19.6%), it was lower in the JE-VAX group (10.7%). All subjects in the pooled 6-months safety population receiving JE-VAX originate from study IC51-301, in which medically attended TEAEs were not collected. In contrast, only 802 subjects (22.6%) of the IXIARO group in the pooled 6-months safety population originate from IC51-301 or IC51-304 where medically attended TEAEs were not collected.

TEAE considered to be treatment related are listed in Table 17.

Table 17: Common Treatment-Related Treatment-Emergent Adverse Events

TEAE system organ class and preferred term[1]	IXIARO N=3558		JE-VAX N=435		HAVRIX N=65		Placebo N=657	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Any treatment-related TEAE[2]	**1362**	**(38.3)**	**149**	**(34.3)**	**12**	**(18.5)**	**255**	**(38.8)**
Nervous system disorders	**707**	**(19.9)**	**80**	**(18.4)**	**7**	**(10.8)**	**135**	**(20.5)**
Headache	684	(19.2)	79	(18.2)	6	(9.2)	132	(20.1)
General disorders and administration site conditions	**626**	**(17.6)**	**73**	**(16.8)**	**2**	**(3.1)**	**119**	**(18.1)**
Fatigue	339	(9.5)	35	(8.0)	1	(1.5)	65	(9.9)
Influenza-like illness	313	(8.8)	30	(6.9)	1	(1.5)	57	(8.7)
Pyrexia	76	(2.1)	10	(2.3)	0	(0.0)	15	(2.3)
Musculoskeletal and connective tissue disorders	**493**	**(13.9)**	**54**	**(12.4)**	**1**	**(1.5)**	**101**	**(15.4)**
Myalgia	478	(13.4)	52	(12.0)	1	(1.5)	94	(14.3)
Gastrointestinal disorders	**195**	**(5.5)**	**29**	**(6.7)**	**1**	**(1.5)**	**40**	**(6.1)**

Nausea	162	(4.6)	26	(6.0)	0	(0.0)	37	(5.6)
Infections and infestations	**66**	**(1.9)**	**4**	**(0.9)**	**4**	**(6.2)**	**10**	**(1.5)**
Nasopharyngitis	28	(0.8)	3	(0.7)	3	(4.6)	4	(0.6)

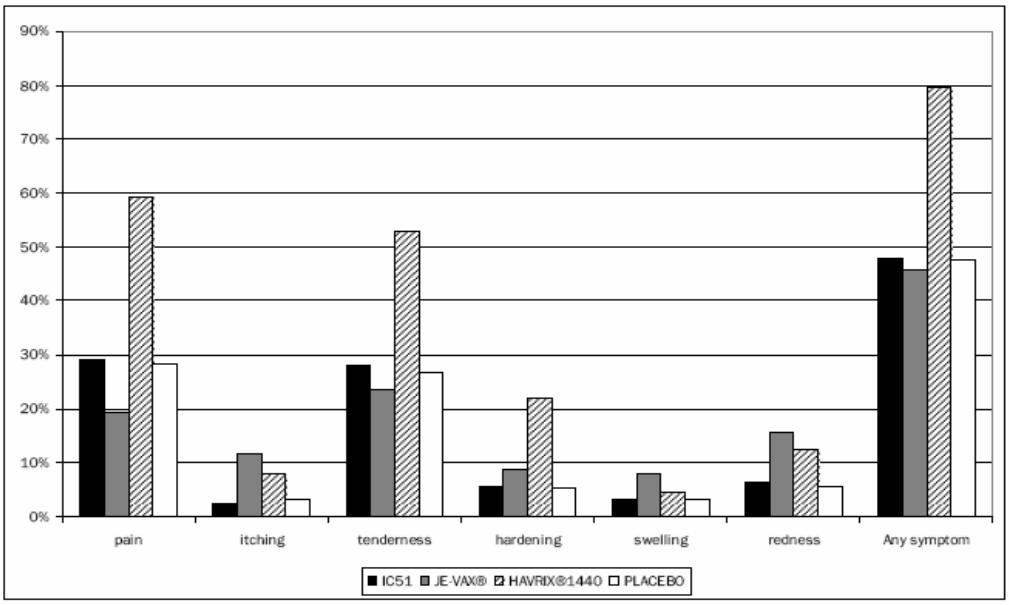
The percentage of subjects reporting treatment-related TEAEs was similar between the IXIARO (38.3%) and placebo groups (38.8%) for all of the most commonly reported events.
The most common treatment-related TEAE by preferred term was headache in each treatment group (IXIARO: 19.2%; placebo: 20.1%; JE-VAX: 18.2%; and HAVRIX: 9.2%). Myalgia was a common treatment-related TEAE for IXIARO (13.4% of subjects), placebo (14.3%) and JE-VAX (12.0%), while nasopharyngitis was commonly reported in the HAVRIX group (4.6%).

Treatment-related severe TEAEs were reported for 2.4%, 2.7%, and 1.4% of subjects in the IXIARO, placebo and JE-VAX groups, respectively. The most common severe treatment-related TEAE was headache in each of these three treatment groups (0.8%, 1.2%, and 0.7% of subjects, respectively).
No related TEAE with fatal outcome was observed in the entire 6 months study period in any vaccination group.

Local Tolerability:
Local symptoms following up to 6 days following vaccination are summarised in Figure 2 and 3.
47.9% of subjects in the IXIARO (IC51) group reported at least one local symptom (pain, itching, tenderness, hardening, swelling or redness) of any severity within the first week after the first vaccination and 29.8% within the first week after the second vaccination on day 28. These proportions were comparable in the placebo group (day 0: 47.6% and day 28: 33.7%) and were higher in the JE-VAX group (day 0: 45.7% and day 28: 42.0%) and in the HAVRIX 1440 group (day 0: 79.7%, no proportion for day 28).

Figure 2:

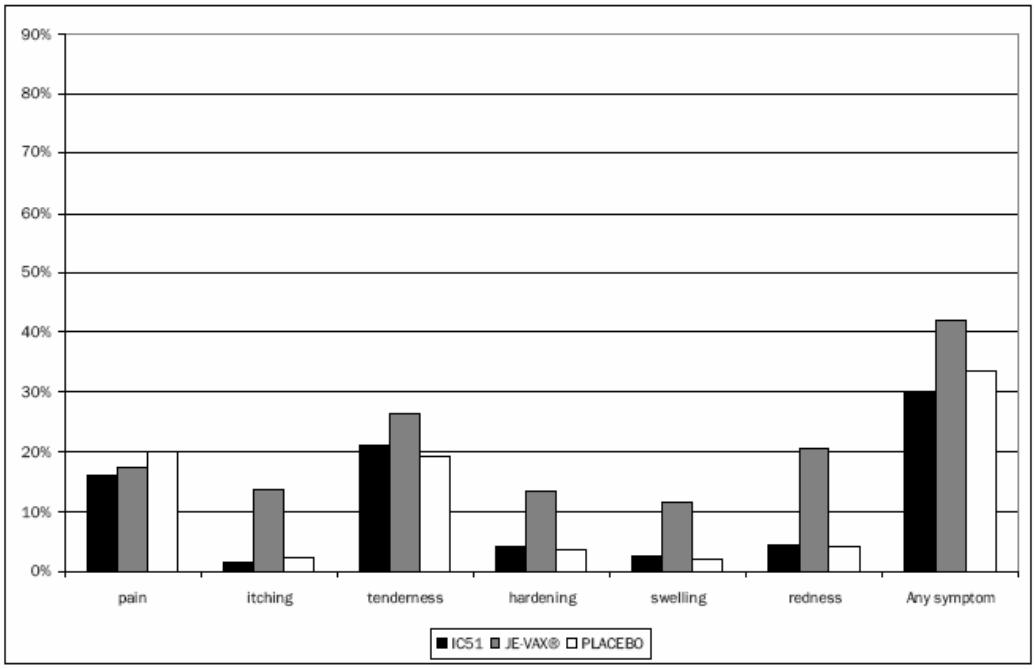
Subjects with a local tolerability symptom up to 6 days after the first vaccination at day 0 (mild, moderate, severe or missing intensity, N=4619)



Percentages are based on the number of subjects within each analysis group, subjects with the same symptom on more than one day counted only once.
IC51 N=3499, JE-VAX® N=411, HAVRIX®1440 N=64, Placebo N=645.
Source: Analysis Table 18, Appendix 6.1

Figure 3:

Subjects with a local tolerability symptom up to 6 days after the second/third (only for study IC51-301) vaccination at day 28 (mild, moderate, severe or missing intensity, N=4532)



Percentages are based on the number of subjects within each analysis group, subjects with the same symptom on more than one day counted only once.
IC51 N=3450, JE-VAX® N=381, Placebo N=701.
Source: Analysis Table 26, Appendix 6.1

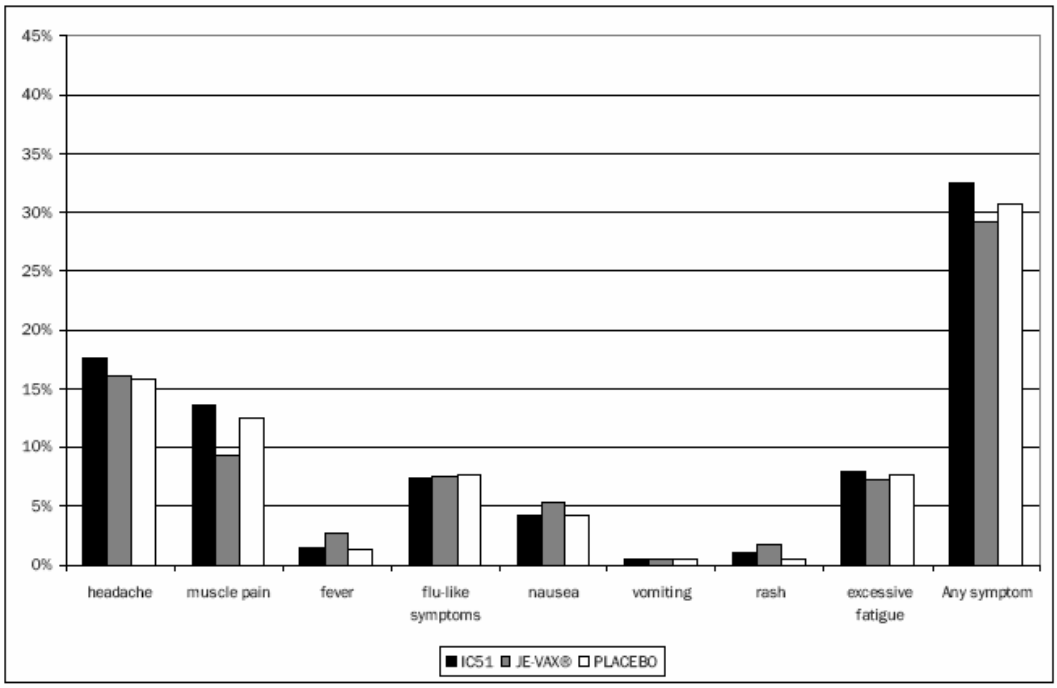
2.5% of subjects in the IXIARO group reported at least one local symptom of severe intensity as judged by the investigator within the first week after the first vaccination and 1.3% of subjects after the second vaccination on day 28. These proportions were comparable in the placebo group (day 0: 2.0% and day 28: 1.3%) and higher in the JE-VAX (day 0: 5.8% and day 28: 10.5%) and HAVRIX 1440 group (day 0: 4.7%, no proportion for day 28).
The analysis of events of special interest (rash, pruritus, conjunctivitis, erythema, dermatitis, hypersensitivity, dyspnoea, circulatory collapse, eye pruritus, flushing, urticaria, hypotension and wheezing) demonstrated an event frequency of 3.5% (95% CI: 2.9% - 4.2%) for the IXIARO group, 5.5% (95% CI: 3.6% - 8.1%) for the JE-VAX group, 4.6% (95% CI: 1.0% - 12.9%) for the HAVRIX 1440 group and 3.7% (95% CI: 2.4% - 5.4%) for the placebo group. The most frequent events were rash and pruritus.

Systemic tolerability:

Systemic symptoms following up to 6 days after vaccination are given Figure 4 and 5.
32.6% of subjects in the IXIARO (IC51) group reported at least one systemic symptom within the first week after the first vaccination and 17.5% within the first week after the second vaccination on day 28.
The corresponding results for the placebo group were comparable (first vaccination: 30.7% and second vaccination: 19.5%) and slightly lower in the JE-VAX group (first vaccination: 29.2% and second vaccination: 12.3%). The most frequent systemic symptoms were headache, muscle pain, flu-like symptoms and excessive fatigue.

Figure 4:

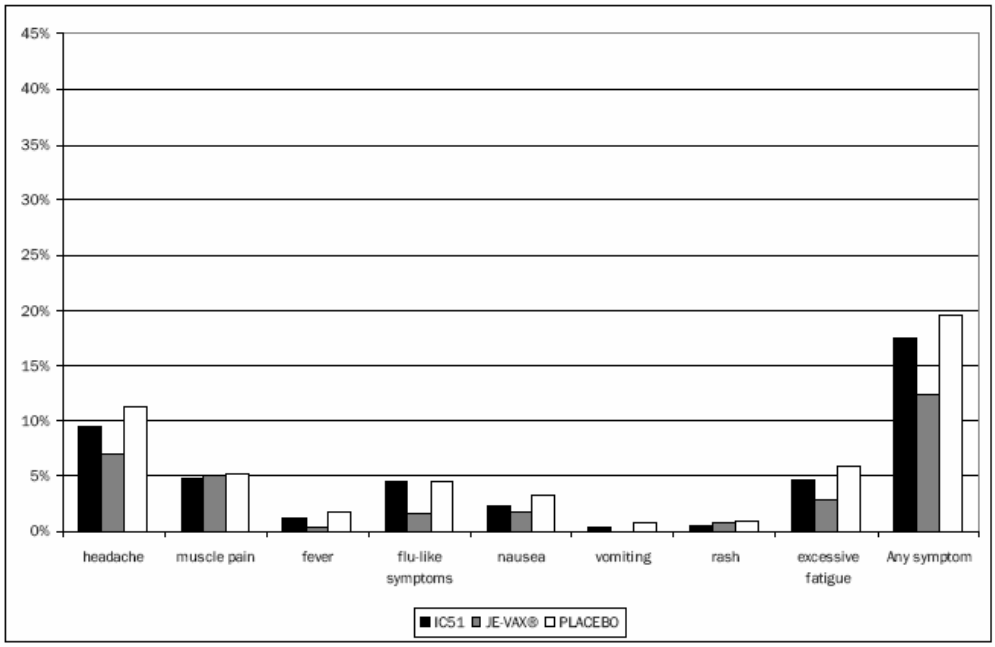
Subjects with a systemic tolerability symptom up to 6 days after the first vaccination at day 0 (N=4429)



Percentages are based on the number of subjects within each analysis group, subjects with the same symptom on more than one day counted only once.
IC51 N=3373, JE-VAX® N=411, Placebo N=645.
Source: Analysis Table 22, Appendix 6.1

Figure 5:

Subjects with a systemic tolerability symptom up to 6 days after the second/third (only for study IC51-301) vaccination at day 28 (N=4343)



Percentages are based on the number of subjects within each analysis group, subjects with the same symptom on more than one day counted only once.
IC51 N=3325, JE-VAX® N=381, Placebo N=637.
Source: Analysis Table 30, Appendix 6.1

Study IC51-303: 12s month follow up of 301 and 302
The 6-months safety analysis included all subjects. Of the 3258 enrolled subjects, 2283 had received IXIARO, 338 had received JE-VAX and 637 had received placebo in the previous studies. The 12-months safety analysis included 180 IXIARO -treated subjects (long-term safety population). The interim analysis of safety following the 12-months visit (Visit 3) was based on the long-term safety population. AEs were summarized that occurred between Visit 1 (Month 2) and Visit 3 (Month 12); AEs that occurred during the preceding studies IC51-301 and IC51-302 were not included in this analysis but have been reported in the respective clinical study reports.
From Month 2 to Month 12 after the first vaccination, 61 (33.9%) of 180 subjects in the long-term safety population experienced at least one AE. Most AEs were mild or moderate in intensity. No AEs were considered to be treatment-related. Three subjects (1.7%) experienced a severe AE and 36 subjects (20.0%) experienced at least one medically attended AE (none considered to be treatment related). No deaths were reported from Month 2 to Month 12 in the long-term safety population.

- Safety in special populations

IXIARO was not investigated in a paediatric population or in pregnant or lactating women. Subjects with certain specified diseases were also excluded from the clinical trials. Excluded have been subjects with a history of flavivirus infection, immunodeficiency, autoimmune diseases, HIV, hepatitis B or C infections, diabetes mellitus, severe cardiopulmonary disorders, acute infections within 4 weeks prior to enrolment or a history of malignancy in previous 5 years.
Vaccination is currently not recommended in these subjects.
A phase II paediatric clinical study (one to three years of age) has been initiated in an endemic population (India) in 2007.
Twenty-nine female subjects in the IXIARO program have become pregnant, 24 of those received IXIARO. Thirteen of the subjects who were vaccinated with IXIARO had live births with normal outcome. Four subjects were considered to have abnormal pregnancy (congenital anomaly of syndactyly, ectopic pregnancy, stillbirth, and miscarriage). Four subjects had elective termination, 2 pregnancies are ongoing and 1 pregnancy has an unknown outcome. On the basis of these data a cumulation of risks can not be discovered.

- Safety related to drug-drug interactions and other interactions

In the clinical study IC51-308 concomitant vaccination with IXIARO and HAVRIX was studied. No effect on the safety or immunogenicity of each vaccine was observed.

- Discontinuation due to adverse events

IC51-302: Seventeen subjects overall experienced TEAEs which led to the withdrawal of study treatment: twelve (0.6%) in the IXIARO group and five (0.8%) in the placebo group. There were two severe TEAEs (gastroenteritis and rash) in the IXIARO group and three severe events in the placebo group (nuchal rigidity, migraine, acute coronary syndrome) that led to withdrawal.
Eight events (two events of headache, influenza like illness, allergic dermatitis, injection site pain, nausea, fatigue and rash) leading to treatment withdrawal in the IXIARO group and one event (nuchal rigidity) leading to withdrawal in the placebo group had a possible or probable relation-ship to study treatment.
IC51-301: TEAEs leading to withdrawal of study drug were experienced by 1.6% subjects (n=7) in the IXIARO group and 1.8% (n=8) subjects in the JE-VAX group. No clinical relevant differences in relationship or severity between the two groups.
There were no withdrawals in the phase I/II studies.
IC51-303: no withdrawals in the ITT population due to TEAEs
IC51-309: Eight subjects (1.3%) overall experienced TEAEs which led to withdrawal of study medication: 4 subjects (1.9%) receiving IXIARO Batch A (rhinitis, neurodermatitis, respiratory tract infection, and nasopharyngitis), 2 subjects (0.9%) receiving Batch B (gastroenteritis and abortion induced), and 2 subjects (0.9%) receiving Batch D (tonsillitis and gastrointestinal disorder). No TEAE

leading to withdrawal was reported by more than one subject. Three events were considered possibly treatment-related; neurodermatitis and nasopharyngitis in the Batch A group, and gastroenteritis in the Batch B group.

- Serious adverse event/deaths/other significant events

IC51-302: A total of 16 subjects who experienced serious TEAEs during the total study period, ten (0.5%) subjects in the IXIARO group and six (0.9%) subjects in the placebo group. No serious TEAEs was considered treatment-related. No deaths occurred in this study.
IC51-301: Only one subject in the IXIARO group experienced a serious TEAE (Myocardial infarction, unlikely to be related, recovered). No subjects died.
IC51-303: During months 2-6: The frequency of SAEs was similar in the placebo group (7 subjects, 1.1%), the IXIARO group (15 subjects, 0.7%) and the JE-VAX group (2 subjects, 0.6%). There was one death in the IXIARO group as a result of a SAE (lung adenocarcinoma metastatic). No SAEs were considered to be treatment-related.
From months 6-12 five additional subjects (2.8%) experienced SAEs. No SAEs were considered to be treatment-related.
IC51-308: No subjects withdrew from the study as a result of a TEAE and no subjects died. One subject in the IXIARO + placebo group (1.5% of the treatment group and 0.5% overall) experienced one SAE. One subject presented with convulsion, a nervous system disorder SAE which was medically attended, moderate in severity and considered unlikely to be related to study treatment.
IC51-309: There were no deaths in this study. There was only one serious TEAE reported during the two months vaccination period. One (0.5%) subject who received Batch D reported a serious TEAE of acute abdomen, which was moderate in severity and judged unlikely to be related to study medication by the Investigator.

- Laboratory findings

There were no safety concerns with regard to haematological parameters across studies.
Of note were increases of liver enzymes (Table 18).

Table 18: Subjects with Clinically Relevant Deviations in Clinical Chemistry Values: Safety Population (Study IC51-302)

	IXIARO N=1993 n (%)	**Placebo N=657 n (%)**
Creatinine Screening	1 (0.1)	0
Day 28	1 (0.1)	1 (0.2)
Day 56	1 (0.1)	0
Potassium Screening	7 (0.4)	2 (0.3)
Day 28	1 (0.1)	1 (0.2)
Day 56	2 (0.1)	0
Calcium Screening	0	0
Day 28	2 (0.1)	0
Day 56	1 (0.1)	1 (0.2)
AST Screening	7 (0.4)	1 (0.2)
Day 28	12 (0.6)	1 (0.2)
Day 56	6 (0.3)	3 (0.5)
ALT Screening	8 (0.4)	2 (0.3)
Day 28	9 (0.5)	3 (0.5)
Day 56	14 (0.7)	3 (0.5)
Alkaline phosphatase Screening	2 (0.1)	0
Day 28	0	0
Day 56	1 (0.1)	0

Bilirubin Screening	7 (0.4)	1 (0.2)
Day 28	5 (0.3)	0
Day 56	5 (0.3)	0

Note: only data for any clinically relevant parameters included. If not clinically relevant in all groups/timepoints then the data (all zero values) have not been included. Abbreviations: N=number of subjects in group; n=number of subjects with event; %=percentage of subjects based on number of subjects in the group; AST= aspartate aminotransferase; ALT= alanine aminotransferase

2.5 Pharmacovigilance

The CHMP considers that the Pharmacovigilance system as described by the Applicant fulfils the requirements and provides adequate evidence that the Applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan

Table: Summary of the risk management plan

Safety concern	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Sensitivity / Allergy Reactions	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Intensified Post Marketing Surveillance within the spontaneous reporting system: Allergy / Sensitivity Questionnaire • Clinical Studies Ongoing follow-up study IC51-303 Ongoing follow-up study IC51-305 Ongoing study IC51-311 Ongoing study IC51-314 • Enhanced Surveillance at Sentinel Sites in 20,000 IXIARO recipients	SPC Section 4.3 Contrainidications: Hypersensitivity to the active substance or to any of the excipients or to any residuals (e.g. protamin sulphate). Individuals who show hypersensitivity reactions after receiving the first dose of the vaccine should not be given the second dose.
Central Neurological Adverse Events	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Intensified Post Marketing Surveillance within the spontaneous reporting system: Neurological Adverse Events Questionnaire • Clinical Studies Ongoing follow-up study IC51-303 Ongoing follow-up study IC51-305 Ongoing study IC51-311 Ongoing study IC51-314 • Enhanced Surveillance at Sentinel Sites in 20,000 IXIARO recipients	
Ossification Delay	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Intensified Post Marketing Surveillance within routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection)	

	• Spontaneous reporting system Pregnancy Questionnaire: specific question	
Thymic Atrophy	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Spontaneous reporting system Pregnancy Questionnaire: unspecific question	
Pediatric Population	• A phase 2 study in pediatric subjects aged 1- 3 years old was done in India. • The pediatric investigational plan for phase 3 development is presently under discussion with the FDA and EMEA Scientific Advice has already been obtained. Phase 3 trials are currently expected to start in 2009. Planning is underway to conduct the pivotal immunogenicity study (IC51-321, comparing IC51 to a locally available comparator in approximately 460 children) and the safety study (IC51-323, approximately 1150 children) in South-East Asia. Additionally, a small immunogenicity study (IC51-322, approximately 50-100 children) will be conducted in western countries.	• SPC Section 4.2 Paediatric: IXIARO is not recommended for use in children and adolescents due to lack of data on safety and efficacy
Vaccination in pregnant women	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Intensified Post Marketing Surveillance within the spontaneous reporting system: Pregnancy Questionnaire and Analysis follow up Programme	• SPC Section 4.6 Pregnancy and Lactation: As a precautionary measure, the use of IXIARO during pregnancy or lactation should be avoided.
Immuno-compromised Individuals	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Individual and cumulative analysis, results reported in PSUR	• SPC Section 4.5 Interaction with other medicinal products and other forms of interaction: In patients receiving immunosuppressive therapy or patients with immunodeficiency an adequate immune response may not be obtained.
Elderly Individuals	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Individual and cumulative analysis, results reported in PSUR	
Co-vaccinated Individuals	• Routine pharmacovigilance (ICSRs, PSURs, monitoring safety profile, safety signal generation and detection) • Individual and cumulative analysis, results reported in PSUR	

The format and content of the RMP and included updates are endorsed. However, the Applicant is committed to provide more detailed information (study synopsis) on the paediatric development plan and for the planned studies IC51-321, -322, and -323 mentioned in the table above.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The Applicant has made considerable progress towards the improvement of critical quality aspects in the production and control of IXIARO. The outstanding major concerns raised during the procedure, which were related to the need to introduce additional relevant quality control procedures and to further develop existing assays, are considered adequately addressed with data and/or follow-up commitments. A number of control measures have been successfully implemented. Some activities related to the quality assurance for IXIARO are still under development and await their final implementation. The Applicant has committed to solve these issues through Follow-up measures.

Non-clinical pharmacology and toxicology

Anti-JE virus neutralizing antibodies have been elicited after vaccination in rats and rabbits. Mice vaccinated with IXIARO are highly protected from lethal challenge with homologous SA14 strain. In addition, in passive protection study, passively transferred human immune anti-sera raised against IXIARO can effectively protect the mice from lethal challenge either with homologous SA14 strain (belongs to genotype III) or with a heterologous strain KE093 (belongs to genotype I). A correlation between the protection and the titre of human neutralising antibodies injected has been shown. However, the definitive $PRNT_{50}$ titre threshold for protection is not demonstrated in the mouse model. Overall, the pharmacodynamic program, including the choice of animal disease model, is considered suitable for pharmacological evaluation of IXIARO, and the studies provide proof of principle, i.e. immunogenicity and protection for this vaccine.

No dedicated single- or repeat-dose toxicity studies or local tolerance studies were conducted and data from histological examinations of vital organs and the injection site are lacking. This is due to the fact that clinical Phase I/II trials had already been completed when the vaccine was taken over by the Applicant This issue of the appropriateness of the preclinical testing programme has previously been raised by the Applicant in a formal procedure.
The Company conducted one GLP reproductive and developmental toxicity study in rats. No treatment related effects were observed with the exception of the incidence of incomplete ossification of parts of the skeleton of the fetuses (4 or more skull bones and ischia) which was statistically significantly higher in one of the two vaccine groups (= in group II, 2 vaccinations) compared to the control group. The Applicant argues that the finding is isolated and not relevant for human use of the vaccine because it was not observed in the other vaccine group (= group I, 3 vaccinations) and no other incomplete development of the fetuses was observed. As no other effects on development of fetuses or birth weights were seen, the finding does not fit in with a general delay in development. However, at present a selective or specific effect of the treatment on ossification rate can not be ruled out. Normally the incomplete ossification is recovered during the postnatal period. However, bone structure was not examined after birth. Therefore, the submitted data do not allow answering whether the incomplete ossification observed is just a delayed event or is persistent in nature.

As requested during the procedure, the Applicant performed histopathological examination on retained samples from F0 lactating rats in the reproductive and development toxicity study, and presented the results. The only finding noted was thymic atrophy, graded as minimal or focal minimal in the Vaccine II group (receiving 2 injections). The Applicant considered the thymic atrophy as being secondary to maternal stress, which could be a reasonable explanation. Since thymic atrophy and foetal incomplete ossification occurred in the same Vaccine group, the Applicant also speculated that maternal stress was a possible cause of abnormal ossification finding. Since thymic atrophy is also reported as isolated and accidental event in other studies the explanation given by the Applicant is acceptable.

Efficacy

In the pivotal clinical study IXIARO demonstrated non-inferiority for genotype III of the JE virus against JE-VAX, a mouse brain derived comparator vaccine. After two doses of IXIARO given within 28 days all vaccinees seroconverted. Moreover, GMTs achieved exceeded those achieved with JE-VAX.

The presented heterologous neutralization data, although limited, together with the review of cross-immunity and distribution among JEV strains are considered sufficient. The applicant will present further analyses of cross-immunity including analysis of neutralising antibodies against all 4 JEV genotypes and a correlation analysis performed on sera from subjects vaccinated with IXIARO and JE-VAX where neutralising antibodies will be measured against the SA 14-14-2 strain, the Nakayama strain, and an additional heterologous virus strain.

Persistence of immunity following primary immunisation, 12 months data from individuals enrolled in non-inferiority study IC51-301 showed decreased GMTs over time. Some individuals dropped below presumed seroprotection levels. Whether this is of clinical significance can only be answered by boostering the study population. Data from ongoing studies will be submitted.

Safety

3310 adults have completed the 6 months follow up visit and a 12 months safety interim analysis with 180 IXIARO treated subjects has been documented. The overall number of included subjects in the phase III studies is acceptable.

The number of adverse reactions or severe, serious or related adverse reactions was similar after administration of IXIARO, JE-VAX or Placebo and did not raise any safety concerns. No related serious adverse reactions have been observed. The most common adverse reactions reported for IXIARO were headache, myalgia, influenza-like illness and fatigue.

No effect was seen on the safety profile of IXIARO compared to placebo (aluminium hydroxide) when split on age groups, sex, or ethnic origin. In the group of elderly only 118 subjects have been evaluated, nevertheless 467 subjects aged 50 - 64 years old have been enrolled showing less related adverse events compared with the younger subjects group. Commonly used drugs for the treatment of coronary heart failure or hypertension do not influence the safety of the vaccine. Receiving immunosuppressive therapy may diminish the immune response. This is mentioned in the SPC.

Regarding local tolerability, IXIARO showed less adverse events than the comparator JE-VAX. Only pain occurred in a higher rate after the vaccination with IXIARO possibly related to the different way of vaccination. IXIARO has been administered i.m. while JE-VAX is used s.c.

Due to a different manufacturing process IXIARO seems to have two advantages compared to JE-VAX regarding safety aspects. Firstly, the absence of the stabilizer gelatine may decrease the probability of serious allergic reactions as well as the late onset of hypersensitivity. Secondly, since IXIARO is manufactured in a cell culture substrate instead of mouse brain tissue, severe neurological adverse effects are not expected following IXIARO vaccination.

Although in the non-clinical reproductive study there were single findings on ossification interferences in one of two vaccine groups these findings are not indicative for an increased risk of malformations. During the clinical studies pregnancy was an exclusion criterion for enrolment, but in spite of that a number of females became pregnant. The Applicant did an extensive survey of the pregnancies with consideration of the week of gestation (29 female subjects have become pregnant, 24 of those received IXIARO). The babies were followed up for three months after delivery. On the basis of these data an accumulation of risks has not been discovered. In addition there is currently no indication of an increased risk from the use of other inactivated JEV vaccines during pregnancy. Considering the non clinical and limited clinical data and according to guideline (EMEA/CHMP/203927/2005) pregnancy

is not a contraindication for the time being but vaccination should be avoided, if possible. A specific pregnancy questionnaire was developed and the Applicant will follow-up pregnant women that have been vaccinated with IXIARO in order to evaluate the risk post marketing.

As stated in the SPC IXIARO may also be administered subcutaneously (s.c.) to patients with thrombocytopenia or bleeding disorders. This is a common practice, however, currently no clinical data recommend such a procedure. The SPC states that there are no efficacy data to support the subcutaneous route.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

- User consultation

The user/readability testing is considered acceptable. The information on user testing provided by the Applicant was found to be satisfactory.

Risk-benefit assessment

Clinical Context

A constantly growing number of people travel to Japanese encephalitis endemic countries either as tourists or on medical or military duty missions. These people are at risk of acquiring a fatal disease during their stay. Also, in recent years sporadic epidemics have been reported from previously non-endemic areas (such as Nepal, Australia) indicating a trend for an ongoing enlargement of regions at risk.

Benefits

The availability of an effective and safe vaccine to protect travelers from Japanese encephalitis is necessary. So far, this demand has been accommodated by "JE-VAX" a Japanese encephalitis vaccine prepared in suckling mouse brains. However production of this vaccine has recently been discontinued and exhaustion of stocks is therefore conceivable. IXIARO is considered qualified to fill the gap originating from "JE-VAX" discontinuation.

Risks

The most common adverse reaction reported for IXIARO were headache, myalgia, influenza-like illness and fatigue but these are not often of severe intensity and the safety profile would not preclude the use of the vaccine.

The current database is considered to be sufficient to describe adverse reactions that occur uncommonly and to give an indication of any rare event. The number of any adverse reactions, or severe, serious or related adverse reaction was similar after administration of IXIARO, JE-VAX or placebo and did not raise any safety concerns. No related serious adverse events have been observed.

Balance

The overall B/R for IXIARO is positive

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

- pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns.

- no additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Ixiaro in the prophylaxis of Japanese Encephalitis for persons 18 years of age and older especially those at risk of exposure through travel or in the course of their occupation was favourable and therefore recommended the granting of the marketing authorisation.