Hepatitis E Vaccine: Composition, Safety, Immunogenicity and Efficacy

A document prepared for Strategic Advisory Group of Experts on Immunization (SAGE) by the Hepatitis E Vaccine Working Group

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Executive Summary

Hecolin®, a recombinant vaccine used for prevention of hepatitis E, contains hepatitis E virus (HEV)-like particles prepared using a recombinant *Escherichia coli* expression system. The vaccine is approved for use in China in people aged 16 and above, and is recommended for individuals at high risk of HEV infection. Hecolin® is well tolerated and has been demonstrated to be safe for use in adults. The main adverse events associated with its use have been local reactions at the injection site.

Current evidence demonstrates that this vaccine is highly immunogenic, with nearly all the recipients seroconverting after three doses administered in a 0, 1 and 6 month schedule. Limited data show that even two doses (at 0 and 6 months, or at 0 and 1 month) lead to a high rate of seroconversion though the antibody titers are lower.

The vaccine protects against symptomatic HEV infection, with a very high efficacy rate. Data on this protection are primarily applicable to genotype 4 disease; data on disease caused by other genotypes are either too limited (genotype 1) or are not available (genotype 2 and 3). The vaccine can effectively lower, but not eliminate, the risk of asymptomatic infection. The duration of follow-up in the available published reports has been for a period of up to nearly 2 years; in addition, some unpublished data for up to 4 years after completing immunization are available. Long-term efficacy beyond this time point, duration of protection, and the need and timing for booster dose remain to be determined. Also, there are no data on protection against severe forms of disease, namely acute liver failure, which is particularly frequent in pregnant women.

Data on safety and efficacy in children or persons aged above 65 years are lacking. Some limited available data suggest that the vaccine is safe in pregnant women; data on immunogenicity and protection specifically in this group are lacking. The vaccine also appears to be safe and immunogenic in hepatitis B carriers; whether these extend to persons with chronic liver disease needs further study. Also, data on protective efficacy in this group are not available. There are currently no data on the use of Hecolin® in children <16 years old, immunosuppressed persons, or its efficacy in protecting against genotype 2 or 3 HEV infection. Further, the efficacy of the vaccine when administered in a post-exposure setting or in controlling disease outbreaks has not yet been studied. Data on these aspects should help better determine the clinical and public health applications of this vaccine.

Background - Hecolin®

Hecolin®, a recombinant vaccine used for prevention of hepatitis E, has been developed and is manufactured by Xiamen Innovax Biotech Co., Ltd. in Xiamen, China. Hecolin® (generic name: Recombinant Hepatitis E Vaccine [*Escherichia coli*]) is prepared using a genetically engineered strain of *E. coli*. It was granted a marketing authorization in China by the then State Food and Drug Administration (SFDA; now China Food and Drug Administration) in December 2011. It has been on the Chinese market since October 2012, where it is approved for use in people aged 16 years and above, and is recommended for individuals at high risk of hepatitis E virus (HEV) infection, including those involved in animal husbandry, food handlers, students, members of the armed forces, women of childbearing age, as well as travellers to endemic areas (http://www.innovax.cn/en/pro1.aspx?CateID=52#103).

This document reviews the data on composition, safety, immunogenicity and clinical efficacy of this vaccine. The data included in this review were derived from articles identified in a search of several databases [MedLine OvidSP (1946-present), EMBASE, Cochrane CENTRAL, PubMed (1946-present) and ICTRP], using the following search terms: 'Hepatitis E Vaccin*', 'Hecolin', 'Virus-like particle', 'Hepatitis E/immunology', 'Hepatitis E/prevention and control' and 'Hepatitis E virus/immunolog*'. The search was limited to human studies. No language restrictions were used. Titles and abstracts of articles identified during the literature search till June 21, 2014 were screened as per the inclusion and exclusion criteria highlighted in Box 1. In addition, the manufacturer of the licensed vaccine was contacted to obtain all the published and unpublished information about studies done with their vaccine.

Box 1: Inclusion and exclusion criteria applied

Inclusion criteria

- Articles that include research on Hepatitis E Vaccine, specifically Hecolin® or *Escherichia coli* expressed HEV 239 VLP recombinant protein vaccine
- Clinical Trials and Post Licensure Studies
- Populations: All
- Languages: No language restriction

Exclusion criteria

- Not Hepatitis E Vaccine or Hecolin® Vaccine
- Non-Human studies
- Pre-clinical studies, systematic review, non-peer reviewed papers, and grey literature

The search strategy identified a total of 3617 records. After initial screening using article titles, 85 articles were included for abstract and full-text assessment. Of these, six articles met the inclusion criteria and were included for analysis.

Composition

Hecolin® is based on a 239 amino acid long recombinant HEV peptide, termed HEV 239, corresponding to amino acids 368-606 of open reading frame 2 (ORF2) which encodes the capsid protein of HEV. The amino acid sequence is derived from a genotype 1 Chinese HEV strain (Li et al., 2005a). HEV 239 is expressed in *Escherichia coli*, where it forms inclusion bodies. The recombinant antigen is then purified using Triton X-100 and urea, and dialyzed against phosphate buffered saline, to enable renaturation of the antigen, followed by further purification by gel filtration high performance liquid chromatography (Li et al., 2005b). The

peptide forms a homodimer and assembles into ~23 nm particles. These dimeric particles have surface protrusions that correspond to a protruding domain on the surface of the HEV capsid that is believed to be responsible for eliciting neutralizing antibodies. Monoclonal antibodies 8C11 and 8H3, previously shown to neutralize HEV infectivity in rhesus macaques (*Macaca mulatta*), have been shown to bind to the dimeric form of HEV 239 as do convalescent sera from patients with hepatitis E. HEV 239 is immunogenic in mice as well as in Rhesus macaques (Li et al., 2005a). Furthermore, HEV 239 has been shown to induce a vigorous T cell response in mice (Wu et al., 2007).

In challenge studies in rhesus macaques, aluminium hydroxide-adjuvanted HEV 239 provided protection against infection by both homologous (genotype 1) and heterologous (genotype 4) strains of HEV with an intravenous challenge of 10^4 genome equivalents (Li et al., 2005a). In the vaccinated animals challenged with a larger HEV inoculum of 10^7 genome equivalents, viral infection occurred in some animals but hepatitis (elevation of liver enzymes) was completely prevented.

Following human clinical trials and licensing, HEV 239 was given the trade name Hecolin®. Each 0.5 ml dose of Hecolin® contains 30 µg of purified recombinant HEV antigen, sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, 0.8 mg aluminium hydroxide, 25 µg thiomersal, and water for injection. The product is a white suspension with each dose of vaccine supplied in a pre-filled syringe (one per package, which measures 13.2 cm X 3.7 cm X 2.15 cm = 100 cm^3); the syringe does not get disabled after use. As per the manufacturer's recommendations, the vaccine should be stored at 2°C to 8°C, out of direct sunlight, and has an approved shelf life of 36 months under appropriate storage conditions. The vaccine is stable for at least 45 months. The product is not yet approved for packaging as multi-dose vials (unpublished data; Innovax).

Analysis of Hecolin® bulks as well as the final product revealed that the vaccine was stable for when stored at 30 to 37°C for two weeks. Higher temperatures and longer incubation periods have not been investigated (unpublished data, Innovax).

Consistency of the Hecolin® manufacturing process has been evaluated by determination of vaccine antigenicity in mice using the final aqueous product. A sandwich ELISA, utilizing capture monoclonal antibodies 8C11 and 1B7, demonstrated very similar relative potencies of 5 commercial scale manufacturing lots of Hecolin® (Wei et al., 2014). Virus-like particles, determined by a variety of methods, remained a consistent size (20-30 nm) during scale-up of the fermentation and for over 30 lots prepared on the commercial manufacturing scale; other parameters such as thermal stability and antigenicity were comparable between vaccine lots (Zhang et al., 2014).

The company has a bulk production capacity of 20 million doses annually; however, currently they have ability to fill/package 5 million doses annually (unpublished data, Innovax). The lead time for production of a new batch (with 2 million doses) is about 6 months (unpublished data, Innovax).

Safety

The safety of Hecolin® has been evaluated during the preclinical as well as the clinical stages of its development. Preclinical toxicity of HEV 239 was evaluated according to Chinese regulatory requirements. This included evaluation of intramuscular irritation of HEV 239 in rabbits; of acute and developmental toxicity in mice and of immunotoxicity in rats (Zhang et al., 2013).

The safety of HEV 239 in humans was evaluated throughout the pre-licensing clinical trials (phase I-III) and in retrospective cohort post-marketing studies (Table 1).

Phase I study

In the phase I safety study, HEV 239 was well tolerated with no vaccine-related serious adverse events (SAEs) in any of the 44 adult subjects each of whom received two 20-µg doses, by intramuscular administration, separated by one month. There was no evidence of clinically significant changes in liver or kidney function 30 days following the second dose of HEV 239 (Zhang et al., 2013).

Phase IIa/b trial

The phase II study had two parts, namely IIa and IIb (Zhang et al., 2009). The phase IIa study investigated safety and dose scheduling of HEV 239 vaccine in 457 anti-HEV negative adults, aged 16-55 years, from a rural area in southern China. The subjects were randomized (ratio of 1:1:1) into three groups; the first received two 20-µg doses of vaccine at 0 and 6 months; the second received three 20 µg doses at 0, 1 and 6 months; the third group received as placebo a licensed hepatitis B virus (HBV) vaccine containing 5 µg of hepatitis B virus surface antigen (HBsAg) at 0, 1 and 6 months. The subjects received 0.5 ml volumes of the HEV vaccine, and an equivalent volume of the HBV vaccine control i.e. a standard dose. The phase IIb part of the study investigated dose escalation and included 155 high school students, aged 16 years and above, randomized into four groups (in ratio of 2:2:2:1), that received three 10, 20, 30 or 40-µg doses administered at 0, 1 and 6 months (Zhang et al., 2009).

After receiving each dose of the vaccine, subjects were observed on site for at least 30 minutes with daily visits thereafter for 3 days. Adverse events (AEs), local and systemic, were recorded by physicians who were not aware of the type of vaccine received. AEs occurring within 30 days of vaccination were documented and subjects with symptoms lasting more than 3 days were visited daily until resolution. Severity of AEs was graded using guidelines issued by the SFDA with grade 3 defined as follows: pain, headache and fever defined as preventing normal activities; redness/swelling at the injection sites exceeding a diameter of 50 mm; and fever $\geq$ 39°C (Zhang et al., 2009).

The vaccine was well tolerated with only mild local AEs, i.e. itching/swelling at the injection site, and occasional fever; no subject had local or systemic AEs of greater than grade 3 in severity. The rate of local reactions was similar between the vaccine dosages, but was higher in individuals who received HEV 239 compared to the control group (Table 2); it was proposed that this was due to the higher protein content of HEV 239 compared to the hepatitis B vaccine control (Zhang et al., 2009).

Phase III trial

The phase III, double-blinded, placebo controlled trial evaluated the efficacy and safety of HEV 239 in 112,604 subjects from 11 townships in the Jiangsu Province, China (Zhu et al., 2010). Healthy adults, aged 16-65 and of either gender, were randomized to receive three doses of HEV 239 or three doses of a placebo – a licensed HBV vaccine, at 0, 1 and 6 months. Of the 56,302 subjects in each group, 48,693 received all three 30-µg doses of HEV 239 whereas 48,663 subjects received all three doses of the placebo.

After each vaccine dose, subjects were observed for at least 30 minutes. They were also requested to report any AEs within 1 month of vaccination. Hospital records and deaths of any

trial subjects were also reviewed. The classification of adverse events followed the definitions set out in the ICH endorsed Medical Dictionary for Regulatory Activities.

Active surveillance of AEs was performed by following a reactogenicity subset of subjects from one township; this subset comprised 1,316 and 1,329 subjects in the HEV 239 group and the placebo group, respectively. Subjects in this subset were visited at home at 6 h, 24 h, 48 h, 72 h, 7 days, 14 days and 28 days after every dose, and any AEs were recorded. There were more local AEs in the HEV 239 vaccinated group than the placebo group i.e. 13.5% vs. 7.1% ($p<0.0001$), respectively. AEs were mainly pain and swelling with itching at the injection site. The rate of systemic AEs was similar in the HEV 239-vaccinated and the placebo groups (20.3% vs. 19.8%, respectively). AEs of grade 3 or more were reported only very rarely, and included injection site swelling in 2 subjects in the HEV 239 vaccine group compared to 1 in the placebo group, fever in 6 individuals in the HEV 239 group compared to 3 receiving placebo, and headache or fever in 2 subjects in the HEV 239 vaccine group compared to none who received placebo (Zhu et al., 2010).

In the total cohort (excluding the reactogenicity subset), the rate of solicited local adverse events occurring within 72 hours of each dose was 2.8% and 1.9% for the vaccine and placebo groups, respectively. Both groups had the same rate of solicited systemic adverse events occurring within 72 hours of each dose (i.e. 1.9%).

For the total vaccinated cohort, there was no significant difference in the rates of unsolicited or serious adverse events for the two groups within 30 days of vaccination with each dose (Table 3). Similar rates of adverse events were observed for the two vaccinated groups up to 19 months (Table 3).

The rates of hospitalization and death among the study subjects in the two groups during the study period were similar (Table 3); none of these events was determined by the Data Safety Monitoring Board to be related to vaccine administration. The study was adequately powered for detection of rare AEs, i.e. a power of 85% to detect an event whose rate in the vaccine group was 0.03% and rate ratio was 5·0 (Zhu et al., 2010).

Data collected during an extended follow up period of between 19 to 55 months from the first vaccine dose (i.e. between 1 year to 4 years after completion of vaccination), showed the number of reported SAEs (4792 vs 4667; $p=0.179$) and the number of subjects with one or more SAE (4602 vs 4490; $p=0.221$) to be comparable between the vaccine and placebo groups (unpublished data, Innovax). The number of deaths over this extended period was nearly 10% higher in the vaccine recipients (408 of 56302) when compared to the placebo recipients (370/56032); however, the difference was not statistically significant ($p=0.172$) (unpublished data, Innovax).

Phase III retrospective cohort study - pregnant women

Following completion of the phase III clinical trial (Zhu et al., 2010), it was found that 37 women in the HEV 239 vaccine group (out of 31,791) and 31 women in the placebo group (out of 31,735) were either pregnant upon commencement of the study or became pregnant during the trial, even though pregnancy was an exclusion criterion for this study. Data for this group of subjects were reviewed carefully (Wu et al., 2012a).

The 37 women in the HEV 239 vaccine group had received 53 vaccine doses (22 single doses, 14 double doses and one triple dose). The vaccine was well tolerated in the pregnant women with only one woman reporting grade 1 inoculation site pain. The rate of AEs was similar in the pregnant women who had inadvertently received HEV 239 vaccine and the

vaccinated non-pregnant women. Half (19; 51.3%) of the pregnant women in the HEV 239 group underwent elective abortion; the rate was 45.2% in the placebo group. No spontaneous abortions occurred in the vaccine group and the remaining 18 babies, delivered either by normal vaginal delivery (n=7) or caesarean section (n=11), were as healthy as those in the control group (vaginal delivery n=7; caesarean delivery n=10); none of the babies had any congenital abnormality. Birth weights (3573.5±356.7 g vs. 3565.6±531.6 g), lengths (50.7±1.3 cm vs. 50.8±1.5 cm) and gestational ages (276.2±7.6 d vs. 276.6±7.1 d) of the babies born to the mothers in the vaccine group and the placebo group were comparable.

Phase III retrospective cohort study: HBsAg-positive individuals

Superinfection with HEV is a risk in chronic liver disease patients, and given the high rates of hepatitis B in China, a look back was performed to review the safety and immunogenicity of HEV 239 in HBsAg-positive subjects from the phase III study (Zhu et al., 2010, Wu et al., 2013). Blood was available from subjects from two townships before and after vaccination with either HEV 239 or the placebo. Of the 14,065 subjects from the two townships, 830 (5.9%) were positive for HBsAg at the onset of the trial; none had evidence of chronic liver disease.

Rates of AEs were similar in HBsAg-positive and HBsAg-negative individuals that received HEV 239. The rate of AEs was higher in the HEV 239 group than in the placebo group, irrespective of HBsAg status (Wu et al., 2013).

Data from post-marketing surveillance

Nearly 200,000 doses of the vaccine have been distributed in the private market in China since the vaccine was licensed; the actual number used and indications are not known. There has been only one report of local adverse event during such use (unpublished data, Innovax), though the completeness of reporting is unclear.

Forthcoming data

A phase 4 trial on nearly 400 elderly (>65 years) persons is currently underway. In that study, a group of elderly subjects have been divided into two groups i.e. anti-HEV seronegative or seropositive, with the former group receiving the usual 3-dose course of Hecolin® at 0, 1 and 6 months whilst the latter group receiving no intervention. All 400 elderly subjects have been requested to report any AEs that occur during the first, second and sixth months post entry. As an immunogenicity control, a further 200 subjects (aged from 16-65) have received 3 doses of Hecolin® according to the normal schedule. This study should provide additional safety data as well as information on immunogenicity of Hecolin® in older persons. Hecolin® is also being used as a placebo in an ongoing phase 3 trial of HPV16/18 vaccine among about 7,300 healthy women aged 18-35 years (randomization ratio = 1:1).

Safety: overall summary

Based on evidence from the phase I, II and III trials conducted by the manufacturer, Hecolin® was well tolerated and demonstrated to be safe for use in healthy adults, with main AEs being local reactions at the injection site. There are limited data on safety of Hecolin® on maternal and fetal outcomes following use during pregnancy, and none for its use among organ transplant recipients, other immunosuppressed persons or persons with chronic liver disease.

Global Advisory Committee on Vaccine Safety (GACVS) reviewed the safety of Hecolin® in its meeting held in June 2014 (World Health Organization, 2014), and concluded as follows:

In summary, available safety data on Hecolin® derived from Phase 1, 2 and 3 clinical trials in healthy subjects are reassuring. However, GACVS noted that there are no safety data in paediatric subjects (<16 years of age), the elderly (>65 years of age), persons with underlying diseases or conditions such as those who are immunosuppressed persons or have liver disease and thus recommended that studies be conducted to assess the safety of Hecolin® in these subpopulations. Any follow-up of those inadvertently vaccinated in pregnancy during the HPV trial should be useful to assess safety in this group. The committee also noted that there are as yet no studies to evaluate the safety and immunogenicity of Hecolin® when given concomitantly with other vaccines. In addition, GACVS recommended that a Phase 4 post-marketing study be conducted once the vaccine is in more widespread use to further assess the safety profile of Hecolin®, in particular with regard to serious and rare adverse events.

Immunogenicity

The immunogenicity of the HEV 239 vaccine in humans has been studied in a phase II and a phase III study.

Phase II study

In this immunogenicity study, HEV 239 was studied in healthy persons aged 16-65 years (Zhang et al., 2009). It had two parts: a dose-scheduling component to determine the optimum number of vaccine doses and a dose-escalation component to determine the optimum dose of the vaccine.

The phase IIa (dose-scheduling) study included 457 seronegative subjects who were randomly assigned to receive intramuscular injection of either two 20-μg doses at months 0 and 6, or three 20-μg doses at months 0, 1 and 6. The subjects assigned to the control group received intramuscular injections of three 5-ug doses of hepatitis B vaccine at months 0, 1 and 6. Immunogenicity was assessed using seroconversion rates and geometric mean concentrations (GMC) of anti-HEV, using the Wantai anti-HEV assay (Wantai Biologic Pharmacy Enterprise, Beijing, People's Republic of China), which uses the dimeric E2 antigen which retains the conformational epitopes present in HEV virions and in the HEV 239 used for HEV vaccine (Zhang et al., 2012). Subjects in both the intervention arms and the control arm of the trial were followed-up at months 0, 2, 6, 7 (one month after the third vaccine dose) and month 13. Serum samples were obtained at these times for the determination of concentration of IgG antibody against HEV (Zhang et al., 2009). The HEV 239 vaccine recipients achieved 98% and 100% seroconversion rates after two and three doses, respectively, compared to only 8% seroconversion rate in the control group. The geometric mean antibody titer levels induced by the three dose regimen were two-fold higher than those induced by the two-dose regimen (geometric mean titer = 15.9 World Health Organization units (Wu)/ml [95% confidence interval (CI) 13.8-18.2] versus 8.6 (95% CI: 6.5-11.3) Wu/ml.

In the phase IIb (dose-escalation) component, subjects received three doses each of 10, 20, 30, or 40-μg (at 0, 1, and 6 months). In this study, the antibody titers induced in previously seronegative young adults (aged 16-19 years) by the three-dose vaccine regimen progressively increased from 10.1 to 23.4 Wu/ml as the amount in each vaccine dose increased from 10-μg to 40-μg, but the differences among the three highest dosages did not reach statistical significance. The GMC of anti-HEV in the vaccinated groups (15.9 Wu/ml) were lower than that in serum

samples of patients with history of hepatitis E (43 Wu/ml), but higher than that in healthy persons who were HEV seropositive, but lacked prior history of hepatitis (0.76 Wu/ml).

Phase III study

In a large phase III study using this vaccine (Zhu et al., 2010), serum samples were taken from a subset of 11,165 subjects (the immunogenicity subset) before vaccination and 1 month after receipt of the third dose. Of the 5567 subjects in this subset in the HEV 239 vaccine group (nearly half of them were anti-HEV seropositive at enrollment), 5494 (98·7%) had a four-fold or greater increase in antibody concentration following vaccination. GMC of anti-HEV in these subjects rose from 0·14 Wu/ml to 19·0 Wu/ml (95% CI: 18·6–19·4). By contrast, 119 (2·1%) of 5598 subjects in the placebo group showed an antibody response; all these subjects were believed to have had subclinical HEV infection. Subsequently, in the phase III clinical trial discussed above, the immunogenicity of the vaccine in persons with pre-existing chronic hepatitis B infection included in this trial was analyzed (Wu et al., 2013). At month 7, the subjects who were HBsAg-positive or HBsAg-negative at baseline showed similar anti-HEV seroconversion rates (98.38% and 98.69%, respectively), and post-vaccination anti-HEV IgG titers (19.32 Wu/ml [95%CI, 17.68–21.12] and 19.00 Wu/ml [95%CI 18.59–19.42], respectively). Antibody dynamics after vaccination were similar for HBsAg-positive and HBsAg-negative subjects, regardless of their baseline anti-HEV status.

The immunogenicity of the vaccine has not yet been evaluated in persons younger than 16 years and older than 65 years of age, or in populations at risk for severe hepatitis E disease, such as persons with chronic liver disease, pregnant women, persons with immunodeficiency states (e.g. HIV/AIDS), and transplant patients receiving immunosuppressive therapy (Crum-Cianflone et al., 2012; Davern et al., 2011; Kamar et al., 2012). The immunogenicity of the vaccine when administered by subcutaneous or intradermal routes, or in an accelerated regimen, e.g., 0, 1 and 2 months has not been studied. There are no data on immunogenicity of this vaccine when co-administered with another vaccine.

The anti-HEV antibodies induced by the vaccine decline with time, but remain detectable up to 4.5 years after the first dose. Persons who were anti-HEV antibody positive at entry into the trial achieved higher antibody titers post-vaccination, and had a slower antibody titer decline over time; in these individuals, an increase in antibody titer and a slow decline of titer post-vaccination was seen even if they had received only one or two of the recommended three doses (unpublished data, Innovax).

The interpretation of data on immunogenicity and persistence of anti-HEV antibody are made difficult by lack of information on protective antibody titer against HEV infection.

Efficacy

Vaccine efficacy is defined as proportional reduction in the incidence of disease among people who have received a vaccine compared to that in a similar group of people who have not been vaccinated, under optimal conditions, such as in a randomized controlled trial. It refers to the clinical benefit provided by a vaccine in the ‘best case scenario’, and differs somewhat from ‘effectiveness’ which relates to ability of vaccine to prevent outcomes of interest in the ‘real world’ situation.

Efficacy of Hecolin® has been assessed to a limited extent in a randomized phase II study (Zhang et al., 2009) and in more detail in a phase III randomized clinical trial (Zhu et al., 2010).

Phase II study

In a phase II dose-scheduling study mainly directed at studying safety, immunogenicity and optimum dose-schedule of the HEV 239 vaccine, occurrence of new HEV infections was studied as one of the secondary outcomes (Zhang et al., 2009). This was done by following up the study subjects, who received three 20-µg doses of HEV vaccine (at 0, 1 and 6 months; group A, n=155), two 20-µg doses of HEV vaccine (at 0 and 6 months; group B, n=151) or three doses of a hepatitis B vaccine (at 0, 1 and 6 months; group C, n=151), for evidence of HEV infection, by looking for spontaneous seroconversion or a >3-fold rise in the level of IgG anti-HEV antibody in paired sera.

Among 151 control subjects in group C (who received hepatitis B vaccine), 20 had evidence of new HEV infection, including 17 with seroconversion to anti-HEV antibody and three others who showed a >3-fold rise in IgG anti-HEV level (6, 19 and 78-fold, respectively). Among the 306 subjects who received HEV vaccine (groups A and B), 13 had new HEV infections, including three with spontaneous seroconversion and 10 with >3 fold rise in anti-HEV antibody levels between vaccine doses that could not be related to vaccine administration. The detailed results are shown in the Table 4 (adapted from: Zhang, 2009). The frequency of new HEV infections in group A after the second dose, and in groups A and B after the completion of vaccination, was significantly lower than that in the control group, suggesting that administration of two or more doses of the vaccine may have prevented new HEV infections. However, none of the 20 persons in the control group and 13 vaccine recipients who had seroconversion reported any hepatitis-like illness.

Phase III randomized field trial

This study included a total of 112,604 healthy adults aged 16-65 years residing in Jiangsu Province of China, who were randomly assigned to receive three 30-µg doses of HEV 239 vaccine (adsorbed to aluminium hydroxide) or a placebo (hepatitis B vaccine) administered intramuscularly at 0, 1 and 6 months (Zhu et al., 2010). Randomization was stratified by age and sex, and the study subjects, care providers and investigators were unaware of the group assignment. Both groups were followed up for 19 months, using an active hepatitis surveillance system comprising 205 sentinels, including 162 community clinics, 30 private clinics, 11 central hospitals located in townships and two central hospitals in one large city, to identify cases with hepatitis. Hepatitis was defined as persons (i) presenting with constitutional symptoms (fatigue, loss of appetite or both) for longer than 3 days and (ii) serum alanine aminotransferase (ALT) exceeding 2.5 times the upper limit of normal. Paired sera were obtained from such subjects at the time of presentation and 2-6 weeks later. The initial serum was tested for markers of infection with various hepatitis viruses, and the paired sera were tested for anti-HEV IgM and IgG, and HEV RNA. For a person with hepatitis to be diagnosed as acute hepatitis E, an additional condition had to be fulfilled, i.e. positive IgM anti-HEV and RNA, or ≥4-times increase in IgG anti-HEV, or both.

Primary efficacy analysis included eligible subjects who had received all three doses of either vaccine (per protocol analysis). The primary endpoint was prevention of hepatitis E (as defined by fulfillment of three conditions, i.e. constitutional symptoms [fatigue, loss of appetite or both] for at least 3 days, serum ALT elevation [of 2.5-fold upper limit of normal range or more], and evidence of HEV infection [positive anti-HEV IgM and HEV RNA, ≥4-times increase in anti-HEV IgG, or both]) in the per-protocol population during the 12 months from the 31st day after the third dose. Efficacy analysis was based on accrued person-time in the

vaccine and control groups, and used an exact conditional procedure under the assumption that the numbers of patients with hepatitis E in the two groups were independent Poisson random variables. In addition, efficacy was also assessed using a Cox proportional hazard model and log-rank test.

In the primary (per protocol) analysis, 15 of the 48,663 placebo recipients (with 48,555.1 person-years at risk) and none of the 48,693 vaccine recipients (with 48,594.6 person-years at risk) developed hepatitis E during the 12 months from the 31st day after the third dose, with a vaccine efficacy of 100% (95% CI = 72.1% to 100%; $p<0.0001$).

An intention-to-treat analysis was also done. It included all eligible subjects who had received at least one dose of either vaccine followed up for 19 months. In this analysis, 22 of the 56,302 placebo recipients and one (with only one vaccine dose) of the 56,302 vaccine recipients had hepatitis E, with vaccine efficacy of 95.5% (95% CI = 66.3% to 99.4%; $p<0.0001$). Another analysis in the same groups for 12 months from the 31st day after the receipt of the final dose (which was the first, second or third dose in those who received one, two or three doses, respectively) revealed 16 cases among placebo recipients and one among vaccine recipients, with protective efficacy of 93.8% (95% CI = 59.8% to 99.9%).

Assessment of efficacy using a Cox proportional hazard model and log-rank test, showed a significant difference between the vaccine and the placebo groups in cumulative incidence of hepatitis E ($p<0.0001$).

An additional analysis evaluated vaccine efficacy after two doses of the vaccine, i.e. in the period between 14 days after the second dose and before the third dose. This revealed five cases among 54,973 placebo recipients (20,196.8 person years) and none among the 54,986 vaccine recipients (20202.1 person years of follow-up) with efficacy of 100.0% (95% CI = 9.1% to 100.0%) (Zhu et al., 2010).

Longer-term efficacy

A subset of subjects in the above phase III randomized trial (residing in two of the 11 rural townships included in the original trial) has been followed up till 25 months after full vaccination course (or 31 months after start of vaccination), to obtain data on longer-term efficacy of hepatitis E vaccine (Zhang et al., 2013). In this report, rates of infection with HEV occurring over the 24 months after vaccination were assessed by comparing the antibody levels in paired serum samples obtained in months 7 and 19 after starting vaccination (i.e. first year after vaccination) and in months 19 and 31 (i.e. second year after vaccination). HEV infection was indicated by a positive anti-HEV seroconversion (when previously seronegative) or by a 4-fold or greater rise in anti-HEV antibody level (when previously seropositive).

Of the 14,094 subjects initially randomly assigned, 14,069 had pre-vaccination antibody testing and were eligible for intention-to-treat analysis. The per-protocol cohort included 12,409 subjects who received all three doses of the HEV vaccine (n=6,176) or placebo (n=6,233); of these, 8,670 subjects (4,322 vaccine and 4,348 placebo recipients) had paired sera taken at months 7 and 19 and 7,478 (3,758 vaccine and 3,720 placebo recipients) had paired sera taken at months 19 and 31. Mean age, gender ratio, baseline anti-HEV IgG seroprevalence and level were comparable between the vaccine and placebo groups.

In the placebo group, 115 subjects had HEV infection, including 98 with positive seroconversion (probable primary infection) and 17 with ≥ 4-fold rise in antibody level (possible re-infection). In the vaccine group, 24 subjects had HEV infection, including 6 with positive seroconversion and 18 with ≥ 4-fold rise in antibody level. Of the total 139 HEV infection

events (115 + 24), only three episodes (all primary infections in control subjects) were associated with clinical illness (hepatitis like symptoms for ≥ 3 days and ALT elevation ≥2.5 times the upper limit of normal) and the remaining 136 were entirely asymptomatic.

The overall per-protocol efficacy was 79.2%, being similar in the first and second year post-vaccination. Overall efficacy in subjects who had received at least one dose of vaccine (intention-to-treat analysis) was 77.0%.

Data from continued follow-up of the original cohorts of vaccinated and unvaccinated persons during the phase III study for 55 months since enrollment (i.e. for 4 years beginning one month after the third dose of vaccine or placebo), showed persistence of protection against hepatitis E with overall protective efficacy of 87% (unpublished data; Innovax).

Efficacy in specific high-risk or other subgroups

No data are available on efficacy of hepatitis E vaccine in high-risk or other special subgroups, such as children (<16 years), elderly persons (>65 years), pregnant women, persons with chronic liver disease, immunosuppressed persons, or persons with other co-existing diseases.

Efficacy against various HEV genotypes

The HEV 239 vaccine is a recombinant protein based on amino acid sequence corresponding to HEV belonging to a genotype 1 Chinese strain. In the large phase 3 trial, of the 23 persons who had HEV infection (22 in placebo group and 1 in vaccine group), viral genotype could be studied in 13 patients (Zhu et al., 2010). Of these 13 isolates (all in placebo group), 12 belonged to genotype 4 and one to genotype 1. This indicates that protection offered by the HEV 239 vaccine was primarily against infection with genotype 4 HEV, a heterologous strain than the one use for developing the vaccine.

There are no data on specific protection offered by the HEV 239 vaccine against genotype 1, 2 or 3 HEV infection, though it is quite likely that it protects against symptomatic infection with these HEV genotypes too. Studies in rhesus macaques have demonstrated protection by HEV 239 vaccine against infection with HEV genotype 1 and 4 (Li et al., 2005a). A cross-genotype conserved neutralizing monoclonal antibody (8G12) binds recombinant E2 peptides from all four HEV genotypes with equivalent affinity, providing indirect evidence of expected cross protection by HEV 239 against HEV genotypes 1-4. In vitro neutralization of HEV infectivity (genotypes 1 and 4) in cell culture has also been shown for 8G12. Further studies have shown that in rhesus macaques, 8G12, prevents disease in animals infected HEV genotypes 1, 3 and 4 (unpublished data, Innovax).

Efficacy in post-exposure setting

No data are available on efficacy of hepatitis E vaccine in the post-exposure setting.

Efficacy in preventing disease transmission

No data are yet available on the effect of HEV vaccination on fecal viral excretion or transmission of HEV infection.

Effectiveness

No real-life effectiveness data on the use of hepatitis E vaccine are available.

GRADE Tables

Following the systematic review of Hecolin®, five key questions concerning the efficacy, safety and immunogenicity of Hecolin® were assessed using evidence summaries and the GRADE approach (www.gradeworkinggroup.org). The questions were as follows:

1a. Efficacy of hepatitis E vaccination in immunocompetent individuals

1b. Efficacy of hepatitis E vaccination in immunocompetent individuals against hepatitis E disease

02. Vaccine safety of Hepatitis E vaccine in immunocompetent individuals

03a. Duration of protection following primary immunization with hepatitis E vaccination in immunocompetent individuals

03b. Duration of protection against disease following primary immunization with hepatitis E vaccination in immunocompetent individuals

GRADE tables (and a listing of data sources on which these are based) for each of these questions are appended at the end of this document.

Tables

Table 1: Summary of safety studies with Hecolin®

Phase	*Aim*	*Number of subjects*	*Adverse events*	*Reference*
I	Safety	44	Well tolerated	(Wu et al., 2012b; Zhang et al., 2013)
IIa	Safety; dose scheduling	457	Well tolerated	(Zhang et al., 2009)
IIb	Safety; dose escalation	155	Well tolerated	(Zhang et al., 2009)
III	Safety; immunogenicity; efficacy	112,604	Well tolerated; no evidence of SAEs in pregnant women or congenital abnormalities in babies born to vaccinated mothers*	(Zhu et al., 2010; Wu et al., 2012a; Wu et al., 2013)

*Inadvertent vaccination of 37 pregnant women who received one or more dose

Table 2: Reactogenicity of HEV 239 vaccine in phase II study

Vaccination		*Subjects*			*Number of doses*	*% Total (grade 3) AE/dose*	
Group	*Dose*	*Number*	*Age Mean ± SD (range)*	*M/F*		*Local*	*Systemic*
Dose schedule	3 x 20 µg HEV 239	155	30.1 ± 12.3 (17-55)	0.67	447	8.5 (1.6)	7.6 (0)
	2 x 20 µg HEV 239	151	32.8 ± 12.5 (17-55)	0.66	286	5.2 (0)	4.9 (0)
	3 x 5 µg placebo*	151	33.6 ± 12.5 (16-55)	0.74	446	2.0 (0)	5.6 (0)
Dose escalation	3 x 10 µg HEV 239	45	18.0 ± 0.62 (17-19)	0.36	132	8.3 (0)	15.2 (0.8)
	3 x 20 µg HEV 239	49	18.0 ± 0.56 (17-19)	0.58	147	6.8 (0)	12.9 (0)
	3 x 30 µg HEV 239	41	17.9 ± 0.66 (17-19)	0.58	121	8.3 (0)	9.9 (0)
	3 x 40 µg HEV 239	20	17.9 ± 0.45 (16-19)	0.67	60	8.3 (1.7)	11.7 (1.7)

AE = adverse event

*Licensed HBV vaccine

Adapted from Zhang et al., 2009

Table 3: Reactogenicity of HEV 239 vaccine in phase III study in the entire vaccinated cohort

Characteristic	*Vaccine group*	*Placebo group*	*p value*
Number of subjects receiving one or more dose	56,302	56,302	
Unsolicited events within 30 days after each dose			
All	6,771 (12.0%, 11.76-12.30)	6,724 (11.9%, 11.68-12.21)	0.666
≥Grade 3	839 (1.5%, 1.39-1.59)	792 (1.4%, 1.31-1.51)	0.241
*SAEs within 30 days after each dose**			
All	284 (0.4%, 0.39-0.50)	245 (0.4%, 0.38-0.49)	0.892
Hospital admission	238 (0.4%, 0.37-0.48)	233 (0.4%, 0.36-0.47)	0.817
Disability	0 (0.0%, 0.00-0.01)	0 (0.0%, 0.00-0.01)	-
Death	10 (0.0%, 0.01-0.03)	12 (0.0%, 0.01-0.04)	0.607
*SAEs during period from month 2 to month 6 and from month 7 to month 19**			
All	1,423 (2.5%, 2.40-2.66)	1,430 (2.5%, 2.41-2.67)	0.894
Hospital admission	1,328 (2.4%, 2.23-2.49)	1,336 (2.4%, 2.25-2-50)	0.875
Disability	0 (0.0%, 0.00-0.01)	0 (0.0%, 0.00-0.01)	-
Death	95 (0.2%, 0.14-0.21)	94 (0.2%, 0.13-0.20)	0.952

Adapted from Zhu et al., 2010.

Data in parenthesis represent (%, 95%CI)

*Data and Safety Monitoring Board did not deem any of the SAEs to be related to vaccination.

Table 4: Efficacy of HEV 239 vaccine in preventing episodes of new HEV infection, as detected by serological testing, in a phase II trial

Subjects		*Observation periods*		*New infection*		
Group	*Number*	*Months of study*	*Person-months*	*Episodes of HEV infection*	*Episodes per 100 person-month*	*Percent efficacy (95% CI)*
During vaccination						
A	128	2-6	512	1	0.20*	86.0 (18.3–99.4)
B	109	1-6	545	10	1.83	--
C	131	0-6	786	11	1.40	--
After vaccination						
A	102	7-12	412	1	0.16*	88.7 (31.0–99.5)
B	78	7-12	468	1	0.21*	85.2 (9.8–99.3)
C	104	7-12	624	9	1.44	--

Adapted from Zhang et al, 2009.

Group A received three 20-µg doses of HEV vaccine (at 0, 1 and 6 months), group B received two 20-µg doses of HEV vaccine (at 0 and 6 months) and group C received three doses of a hepatitis B vaccine (at 0, 1 and 6 months). Episodes of new infection were identified by the spontaneous HEV serological changes observed in consecutive pairs of serum samples taken from group A on months 2 and 6 and on months 7 and 13; from group B on months 1 and 6 and on months 7 and 13, or, in successive pairs of samples taken from group C on months 0,2,6,7 and 13 of the study. (* Significantly lower than control group).

Table 5: Efficacy of HEV 239 vaccine in preventing HEV infection in the phase III trial

Cohorts	*Months post-vaccination*	*Infection rate (episodes/person-year)*		*Efficacy % (95% CI)*
		Vaccine group*	Placebo group*	
Per-protocol	1-24 (overall)	0.30 (24/8080)	1.43 (115/8068)	79.2 (67.7-86.6)
	1-12	0.26 (11/4322)	1.49 (65/4348)	83.0 (67.8-91.0)
	13-24	0.35 (13/3758)	1.34 (50/3720)	74.3 (52.6-86.0)
Intention-to-treat	1-24	0.33 (28/8610)	1.41 (121/8564)	77.0 (65.3-84.7)

Adapted from Zhu et al, 2010.

* In the parenthesis, numerators indicate the number of subjects with infection and the denominators indicate the total number of subjects studied.

GRADE Table 01a. Efficacy of hepatitis E vaccination in immunocompetent individuals

Population : Immunocompetent individuals(**>**16 years)

Intervention: Hepatitis E vaccination (Hecolin®)

Comparison: Non- Hepatitis E vaccination

Outcome : Infection with Hepatitis E

What is the scientific evidence of the efficacy of primary immunization with Hepatitis E vaccination (versus control) to prevent infection with Hepatitis E in immunocompetent individuals?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		3/ RCT 1/ observational[1]	4
	Factors decreasing confidence	Limitation in study design	Serious[2]	-1
		Inconsistency	None serious	0
		Indirectness	Serious[3,4]	-1
		Imprecision	None serious	0
		Publication bias	None serious	0
	Factors increasing confidence	Large effect	Not applicable	0
		Dose-response	Not applicable	0
		Antagonistic bias and confounding	Not applicable	0
	Final numerical rating of quality of evidence			**2**
Summary of Findings	**Statement on quality of evidence**			Our confidence in the estimate of the effect on the health outcome is limited.
	Conclusion			Our confidence in the estimate of the effect is limited that primary immunization with hepatitis E vaccine (HEV) decreases the incidence of hepatitis E infection significantly compared to a control. A phase II trial estimated that after receipt of the 3 HEV doses, the vaccinated groups had a significantly lower percentage of episodes of subclinical Hepatitis E per person month (0.21% and 0.16% vs 1.44%).

[1] A phase IIa randomized controlled trial (RCT) by Zhang et al. 2009(2) reported on the occurrence of new Hepatitis E infection among 457 study subjects by assessing IgG anti-Hepatitis E vaccine (HEV) levels in successive pairs of consecutive serum samples. Within the control group, 20 episodes (17 individuals) of seroconversion and 13 episodes (13 individuals) within the vaccinated group were reported during the study period of 12 months. After receipt of 3 HEV doses, the vaccinated groups had a significantly lower percentage of episodes per person month (0.21% and 0.16% vs 1.44%). Vaccine efficacy was 85.2%(95% CI: 9.8-99.3% using a 2 dose schedule) and 88.7%(95%CI: 31.0-99.5% using a 3 dose schedule). All 33 episodes were subclinical as no study subject revealed a history of Hepatitis E during the trial. Zhu et al. 2010 (3) reported only on clinical 23 hepatitis E cases (22 cases in placebo vs 1 case in the vaccine group) within a large phase III RCT including 122.179 subjects corresponding with an estimated vaccine efficacy within the follow-up period of 19 months of 95.5% (95% CI 66.3-99.4%) within an intention to treat analysis that included everybody having received at least one dose (though most received 3 doses)and assessed a significant difference in incidence ($p<0.0001$) of hepatitis E between placebo and vaccine group. No data on protection against subclinical infection is available. The significant difference for a reduced risk of infection after vaccination (RR= 0.15, 95% Ci 0.3-0.83) was confirmed within a 24-month post-vaccination follow-up RCT of 12 409 subjects from Zhang et al 2013 (4). The estimated vaccine efficacy was 79.2% (95%CI 67.7-86.6) over the 2 year study period. An observational subset of hepatitis b surface antigen positive subjects (Wu et al.2013 (1)) showed no significant difference (98.38% vs. 98.69%, p=.06063) in seroconversion rates to anti-HEV IgG after 3 doses of HEV.

[2] Allocation concealment not clearly stated (Zhang et al. 2009 (2)and Zhu et al. 2010 (3)). The vaccine proved to be efficacious against genotype 1 and 4. The phase III trial was conducted in a region where both genotype 1 and 4 co-circulate. No proved protection against genotype 2 and 3.

[3] Only healthy individuals aged 16- 65 were included, no data available on immunization of children and the immunocompromised. No downgrading for indirectness, as the determined age group in which the vaccine should be used may vary among settings.

[4] The phase III trial provided no data on efficacy against subclinical infection with Hepatitis E.

Reference List[1-4]

1. Wu T, Huang SJ, Zhu FC et al. Immunogenicity and safety of hepatitis E vaccine in healthy hepatitis B surface antigen positive adults. Hum Vaccin Immunother 2013;9:2474-2479.

2. Zhang J, Liu CB, Li RC et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009;27:1869-1874.

3. Zhu FC, Zhang J, Zhang XF et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895-902.

4. Zhang J, Zhang XF, Zhou C et al. Protection against hepatitis E virus infection by naturally acquired and vaccine-induced immunity. Clin Microbiol Infect 2014;20:O397-405.

GRADE Table 01b. Efficacy of hepatitis E vaccination in immunocompetent individuals against hepatitis E disease

Population : Immunocompetent individuals(>16 years)

Intervention : Hepatitis E vaccination (Hecolin®)

Comparison : Non-hepatitis E vaccination

Outcome : Hepatitis E disease

What is the scientific evidence of the efficacy of primary immunization with Hepatitis E vaccination (versus control) to prevent Hepatitis E disease in immunocompetent individuals?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		2/ RCT 1/observational[5]	4
	Factors decreasing confidence	Limitation in study design	Serious[6]	-1
		Inconsistency	None serious	0
		Indirectness	None serious[7]	0
		Imprecision	None serious	0
		Publication bias	None serious	0
	Factors increasing confidence	Large effect	Not applicable	0
		Dose-response	Not applicable	0
		Antagonistic bias and confounding	Not applicable	0
	Final numerical rating of quality of evidence			**3**
Summary of Findings	**Statement on quality of evidence**			We are moderately confident in the estimate of effect on health outcome. The true effect is likely to be close to the estimate of the effect.
	Conclusion			Our confidence in the estimate of the effect is moderate that primary immunization with hepatitis E vaccine (HEV) decreases the incidence of hepatitis E disease significantly compared to placebo. A large phase III trial estimated a vaccine efficacy of 95.5% (95% CI 66.3-99.4%) after at least one dose of HEV.

[5] A phase IIa randomized controlled trial (RCT) by Zhang et al. 2009 (2) reported on the occurrence of new Hepatitis E infection among 457 study subjects by assessing IgG anti-Hepatitis E vaccine (HEV) levels in successive pairs of consecutive serum samples. Within the control group, 20 episodes (17 individuals) of seroconversion and 13 episodes (13 individuals) within the vaccinated group were reported during the study period of 12 months per person years. After receipt of the 3 HEV doses, the vaccinated groups had a significantly lower percentage of episodes per person month (0.21% and 0.16% vs 1.44%). Vaccine efficacy was 85.2%(95% CI: 9.8-99.3% using a 2 dose schedule) and 88.7%(95%CI: 31.0-99.5% using a 3 dose schedule). All 33 episodes were subclinical as no study subject revealed a history of Hepatitis E during the trial. Zhu et al. 2010 (3)reported 23 hepatitis E cases (22 cases in placebo vs 1 case in the vaccine group) within a large phase III RCT (122.179 subjects) and estimated vaccine efficacy within the follow-up period of 19 months to be 95.5% (95% CI 66.3-99.4%) within an intention to treat analysis that included everybody having received at least one dose (though most received 3 doses)and assessed a significant difference in incidence ($p<0.0001$) of hepatitis E between placebo and vaccine group. An observational trial subset of hepatitis b surface antigen positive (Wu et al. 2013(1)) showed no significant difference (98.38% vs. 98.69%, p=.06063) in seroconversion rates to anti-HEV IgG after 3 doses of HEV.

[6] Allocation concealment not clearly stated (Zhang et al. 2009 (2) and Zhu et al. 2010(3)). The vaccine proved to be efficacious against genotype 1 and 4. The phase III trial was conducted in a region where both genotype 1 and 4 co-circulate. No proved protection against genotype 2 and 3.

[7] Only healthy individuals aged 16- 65 were included, no data available on immunization of children and immunocompromised. No downgrading for indirectness, as the determined age group in which the vaccine should be used may vary among settings.

Reference List[1-3]

1. Wu T, Huang SJ, Zhu FC et al. Immunogenicity and safety of hepatitis E vaccine in healthy hepatitis B surface antigen positive adults. Hum Vaccin Immunother 2013;9:2474-2479.

2. Zhang J, Liu CB, Li RC et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009;27:1869-1874.

3. Zhu FC, Zhang J, Zhang XF et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895-902.

GRADE Table 02. Vaccine safety of hepatitis E vaccine in immunocompetent individuals

Population : Immunocompetent individuals (**>**16 years)

Intervention : Hepatitis E vaccination (Hecolin®)

Comparison : Non-Hepatitis E vaccination

Outcome : Serious adverse events following immunization

In immunocompetent individuals, what is the incidence of serious adverse events following immunization (versus control) with any dose of Hepatitis E vaccine?

			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		2/ RCT 3/ observational[8]	4
	Factors decreasing confidence	Limitation in study design	Serious[9]	-1
		Inconsistency	None serious	0
		Indirectness	None serious[10]	0
		Imprecision	None serious	0
		Publication bias	None serious	0
	Factors increasing confidence	Large effect	Not applicable	0
		Dose-response	Not applicable	0
		Antagonistic bias and confounding	Not applicable	0
	Final numerical rating of quality of evidence			**3**
Summary of Findings	**Statement on quality of evidence**			**We are moderately confident in the estimate of effect on health outcome. The true effect is likely to be close to the estimate of the effect.**
	Conclusion			**Our confidence in the estimate of the effect is moderate that incidence of serious adverse events following Hepatitis E vaccination is low. Judged on the Phase I, II and III trials and the reactogenicity subset of the latter study, the rates of solicited adverse events were not concerning. Nevertheless the safety follow-up surveillance was largely passive, only healthy individuals aged 16-65 years were included and the pregnancy safety data was limited.**

[8] Based on encouraging safety assessments in the observational phase I trial (Wu et al. 2012(2)), a phase IIa/IIb randomized controlled trial (Zhang et al. 2009 (4)) with a total of 457 (16-55years) (20 µg at 0,1,6 months or at 0,6 months, Hepatitis B vaccine as control) and 155 subjects (16-19years) (10, 20, 30, 40 µg at 0,1,6 months) indicated no serious adverse events (SAE) following immunization above grade 3 (SFDA Guideline). No significant difference in grade 3 local or systemic reactions between the vaccine group and the control. One large phase III trial (Zhu et al. 2010 (5)) with 112 604 healthy individuals (30 µg at 0,1,6 months) showed no significant difference of adverse events between vaccine and control group within the total cohort. Within a reactogenicity subset including 1645 subjects, solicited local adverse events within 72h after each dose were higher (<0.0001) in the vaccine group (13.5%) than in the placebo group (7.1%). Systemic adverse events were similar for both groups (20.3%vs.19.8%). These findings are reflected within the entire cohort. Safety was confirmed in two observational study subsets: A pregnancy subset- retrospective analysis of the phase III trial (Wu et al. 2012(1)) found no significant difference of SAE in women or their infants when receiving vaccine or placebo. Same applies to an analysis of a subset within the phase III trial of individuals with pre-existing chronic hepatitis B (Wu et al. 2013 (3)).

[9] Allocation concealment not clearly stated (Zhang et al. 2009 and Zhu et al. 2010)

[10] Only healthy individuals aged 16- 55 were included, no data available on immunization of children and immunocompromised. No downgrading as the determined age group in which the vaccine should be used may vary among settings. Limited data on 12 cases of ALT increase excluded from analysis on safety by the DSMB, no downgrading regarding this issue (5).

Reference List[1-5]

1. Wu T, Zhu FC, Huang SJ et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis 8. Hepatology 2012;55(6):2038.

2. Wu T, Li SW, Zhang J, Ng MH, Xia NS, Zhao Q. Hepatitis E vaccine development: a 14 year odyssey. Hum Vaccin Immunother 2012;8(6):823-827.

3. Wu T, Huang SJ, Zhu FC et al. Immunogenicity and safety of hepatitis E vaccine in healthy hepatitis B surface antigen positive adults. Hum Vaccin Immunother 2013;9:2474-2479.

4. Zhang J, Liu CB, Li RC et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009;27:1869-1874.

5. Zhu FC, Zhang J, Zhang XF et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895-902.

GRADE Table 03a. Duration of protection following primary immunization with hepatitis E vaccination in immunocompetent individuals

Population : Immunocompetent individuals (>16 years)

Intervention : Hepatitis E vaccination (Hecolin®)

Comparison : Non-hepatitis E vaccination

Outcome : Infection with hepatitis E virus

What is the scientific evidence of the continuous duration of protection against infection with Hepatitis E following primary immunization with Hepatitis E vaccination (versus control) to prevent infection with Hepatitis E in immunocompetent individuals?				
			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		3/ RCT[11]	4
	Factors decreasing confidence	Limitation in study design	Serious[12]	-1
		Inconsistency	None serious	0
		Indirectness	None serious[13]	0
		Imprecision	None serious	0
		Publication bias	None serious	0
	Factors increasing confidence	Large effect	Not applicable	0
		Dose-response	Not applicable	0
		Antagonistic bias and confounding	Not applicable	0
	Final numerical rating of quality of evidence			**3**
Summary of Findings	**Statement on quality of evidence**			**We are moderately confident in the estimate of effect on health outcome. The true effect is likely to be close to the estimate of the effect.**
	Conclusion			**Our confidence in the estimate of the effect is moderate that primary immunization with hepatitis E vaccine (HEV) decreases the incidence of hepatitis E infection significantly compared to placebo within a period of ≤24 months following immunization though no data is available on the long-term protection following primary immunization with HEV.**

[11] A phase IIa randomized controlled trial (RCT) by Zhang et al. 2009 (1) with 457 study subjects reported a significant difference in hepatitis E episodes after receipt of 3 doses of Hepatitis E vaccine (HEV) within the 12 months study period. A phase III RCT (Zhu et al. 2010 (3)) including 122.179 subjects reported a significant difference in incidence ($p<0.0001$) of hepatitis E between placebo and vaccine group within the follow-up period of 19 months. The significant difference for a reduced risk of infection after vaccination (RR= 0.15, 95% Ci 0.3-0.83) was confirmed within a 24-month post-vaccination follow-up of 12 409 subjects from the Zhang et al 2013 (2) RCT. The estimated vaccine efficacy was 79.2% (95%CI 67.7-86.6) over the 2 year study period.

[12] Allocation concealment not clearly stated (Zhang et al. 2009 (1) and Zhu et al. 2010 (3))

[13] Only healthy individuals aged 16- 65 were included, no data available on immunization of children and immunocompromised. No downgrading as the determined age group in which the vaccine should be used may vary among settings.

Reference List[1-3]

1. Zhang J, Liu CB, Li RC et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009;27(12):1869-1874.

2. Zhang J, Zhang XF, Zhou C et al. Protection against hepatitis E virus infection by naturally acquired and vaccine-induced immunity. Clin Microbiol Infect 2014:20:O397-405.

3. Zhu FC, Zhang J, Zhang XF et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895-902.

GRADE Table 03b. Duration of protection following primary immunization with hepatitis E vaccination in immunocompetent individuals

Population : Immunocompetent individuals (**>**16 years)

Intervention : Hepatitis E vaccination (Hecolin®)

Comparison : Non-hepatitis E vaccination

Outcome : Hepatitis E disease

What is the scientific evidence of the continuous duration of protection against Hepatitis E disease following primary immunization with Hepatitis E vaccination (versus control) to prevent infection with Hepatitis E in immunocompetent individuals?

			Rating	Adjustment to rating
Quality Assessment	No. of studies/starting rating		2/ RCT[14,15]	4
	Factors decreasing confidence	Limitation in study design	Serious[16]	-1
		Inconsistency	None serious	0
		Indirectness	None serious[17]	0
		Imprecision	None serious	0
		Publication bias	None serious	0
	Factors increasing confidence	Large effect	Not applicable	0
		Dose-response	Not applicable	0
		Antagonistic bias and confounding	Not applicable	0
	Final numerical rating of quality of evidence			**3**
Summary of Findings	**Statement on quality of evidence**			**We are moderately confident in the estimate of effect on health outcome. The true effect is likely to be close to the estimate of the effect.**
	Conclusion			**Our confidence in the estimate of the effect is moderate that primary immunization with hepatitis E vaccine (HEV) decreases the incidence of hepatitis E cases significantly compared to placebo within a period of ≤19 months following immunization. No data is available on the long-term protection following primary immunization with HEV.**

[14] A phase IIa randomized controlled trial (RCT) by Zhang et al. 2009 (1) with 457 study subjects reported a significant difference in hepatitis E episodes after receipt of 3 doses of Hepatitis E vaccine (HEV) within the 12 months study period. A phase III RCT (Zhu et al. 2010(2)) including 122.179 subjects reported a significant difference in incidence ($p<0.0001$) of hepatitis E between placebo and vaccine group (22 cases in placebo vs 1 case in the vaccine group) within the follow-up period of 19 months.

[15] Unpublished data reports that within a follow-up period of 55 months of Zhu et al. 2010 (2), vaccine efficacy after 3 doses of HEV was estimated to be 93% (95%CI:79-98%), though these data could not be graded due to lack of publication.

[16] Allocation concealment not clearly stated (Zhang et al. 2009 (1) and Zhu et al. 2010 (2))

[17] Only healthy individuals aged 16- 65 were included, no data available on immunization of children and immunocompromised. No downgrading as the determined age group in which the vaccine should be used may vary among settings.

Reference List[1-3]

1. Zhang J, Liu CB, Li RC et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. Vaccine 2009;27:1869-1874.

2. Zhu FC, Zhang J, Zhang XF et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010;376:895-902.

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