

Official Title: An Open, Pared Trial of Recombinant Hepatitis E Vaccine (Escherichia Coli) Hecolin® in the Chronic Hepatitis B Patients on the Clinical Stability. (Aged 30 Years or Over)

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Brief Study Protocol and Statistical Analysis Plan

Protocol ID: PRO-HE-009

Title: Immunogenicity and safety of Hepatitis E vaccine Hecolin® in persons with chronic hepatitis B: an open-label study in China

Study Dates: Aug, 2016-Dec, 2017

Study Sites: Rushan city, Shandong province, China

Study Objectives

Primary Objectives:

- Evaluate the immunogenicity of the Hepatitis E vaccine in persons with chronic hepatitis B.

Secondary Objectives:

- Evaluate the safety of the Hepatitis E vaccine in persons with chronic hepatitis B.
- Evaluate the safety and immunogenicity of the Hepatitis E vaccine in healthy persons without chronic hepatitis B.

Selection of Study Population

The aim was to enrolled approximately 464 subjects aged ≥ 30 years who were assigned to CHB group and healthy adults group by 1:1. The CHB patients had been diagnosed by clinicians according to the Asian-Pacific clinical practice guidelines before the recruitment.

Inclusion criteria:

all volunteers:

- Aged over 30 years old on the day of enrollment.
- Axillary temperature is below than 37.0 °C.
- No administration of hepatitis E vaccine before the study.
- Judged as healthy and eligible for vaccination by the investigators through a self-reported medical history and some physical examinations.
- Able to understand this study information and willing to comply with all study requirements.

- Willing to participate in this study and sign informed consent form.
- Negative serological markers for hepatitis E.

experiment group:

- ALT < 1.5×ULN.
- No spleen swelling, no cirrhosis and no hepatocellular carcinoma.

Exclusion criteria:

- With clinical evidence of malignant tumor.
- History of severe cardio-cerebrovascular disease.
- Administration of hepatotoxicity drugs before or during the study.
- Pregnancy, breast-feeding or plan to be pregnant in 7 months later.
- Participated in any other clinical trial during the study period.
- Use of any investigational product or non-registered product (drug or vaccine) within 30 days preceding the first dose of the study vaccine or plan to use during the study period.
- Received immunosuppressed, immunoregulation therapy or corticosteroid systemic therapy for more than 14 days in the 6 months before entry, except local treatment.
- Administration of any immunoglobulin or blood products within 3 months preceding the first dose of the study vaccine, or plan to use during the study period.
- Administration of any inactivated vaccines within 14 days preceding the first dose of the study or attenuated live vaccines within 21 days preceding the first dose of the study.
- Had a fever (axillary temperature over 38°C) within 3 days or acute illness requiring systemic antibiotics or antiviral treatment within 5 days before vaccination.
- Immunodeficiency (such as HIV carriers), primary disease of important organs, malignant tumor, or any immune disease (such as systemic lupus erythematosus, arthritis pauperum, splenectomy or functional asplenia or other disease which might affect immune response).
- History of allergic disease or history of serious adverse events occurring after vaccination, i.e., allergy, urticaria, dyspnea, angioneurotic edema or abdominal pain.
- Allergic history to any component of this vaccine.
- Asthma that needed emergency treatment, hospitalization, oral or intravenous corticosteroid to keep stable in the past two years.
- Combining another severe internal medicine disease (such as severe hypertension, cardiopathy, diabetes and hyperthyroidism).
- Abnormal coagulation function or coagulopathy diagnosed by doctor.
- Epilepsy (not including alcohol epilepsy within 3 years prior to abstinence and simple epilepsy that do without curing within 3 years prior to the study).
- Abnormal psychology or mind affecting the individual's ability to obey the study require
- Other medical, psychological, social or occupational factors that, according to the

investigators' judgment, might affect the individual's ability to obey the protocol or sign the informed consent.

Vaccination Schedule

Hecolin® (Xiamen Innovax Biotech Co. Ltd, Xiamen, China) was given intramuscularly to the subjects at day 0, month 1 (+0-30 days) and month 6 (-30-60 days) without any charge. Each dose of vaccine contains 30 µg of the purified antigen adsorbed to 0.8 mg aluminium hydroxide suspended in 0.5 mL buffered saline. All the vaccines have been qualified by China Food and Drug Administration (CFDA) before use.

Safety Assessment

Adverse events (AE) were observed within 30 days after each dose. After each dose, the participants were observed for 30 min for immediate adverse reactions. Within 0-7 days after each dose, the local symptoms at the injection site including pain, red and swollen, pruritus, indurate, and the systematic symptoms including body temperature, allergic reaction, head pain, weakness, nausea, emesis, diarrhea, muscle pain and cough were observed and recorded at the safety diary every day by the participants. AE which happened within 30 days after each dose and drugs and other vaccines administrated were also recorded at the safety diary. All serious adverse events (SAE) were required to report to the researchers within 24 hours during the whole study. The participants who reported AE would be followed up at the end of the study (month 7).

Laboratory Test

Blood samples were obtained from each participant right before the 1st dose (day 0), one month after the 1st dose (at before the 2nd dose, month 1), at before the 3rd dose (month 6) and one month after the 3rd dose (month 7). Five indicators including anti-HEV IgM (only at day 0), anti-HEV IgG, ALT, AST and TBIL were detected in both groups; HBV DNA was detected only in the CHB group. Anti-HEV IgM and IgG was qualitatively detected by ELISA method. Quantitative detection was further conducted for those positive for anti-HEV IgG and the result was expressed in World Health Organization (WHO) units per mL(Wu/mL). The lower limit of anti-HEV IgG quantification was 0.064 Wu/mL. For the analysis, the antibody concentration in samples negative for anti-HEV IgG was arbitrarily set at 0.032 Wu/mL. All reagents for anti-HEV IgG and IgM were supplied by Beijing Wantai Biological Pharmacy Enterprise, Beijing, China and all detections were completed by staffs at Xiamen Innovax Biotech Co. Ltd. ALT and AST were detected by rate method, TBIL was detected by exploring bromocresol green method and HBV DNA was completed by Realtime PCR by the third-party testing organization (ADICON Clinical Laboratories, INC).

Statistical Considerations

Primary endpoints:

- Number of participants whose anti-HEV antibody seroconverted at month 7.

- Geometric mean concentrations of anti-HEV antibody at month 7.

Secondary endpoints:

- Number of participants with changes in liver function index before and one month after the first dose.
- Number of participants with changes in liver function index before and one month after the third dose.
- Number of participants with changes in liver function index before the first dose and one month after the third dose.
- Number of participants who experienced any adverse reactions/events during the whole period of observation (0-7 months).
- Number of participants who experienced solicited adverse reactions/events within 7 days after each vaccination.
- Number of participants who experienced solicited local adverse reactions/events within 7 days after each vaccination.
- Number of participants who experienced solicited system adverse reactions/events within 7 days after each vaccination.
- Number of participants who experienced unsolicited adverse reactions/events within 30 days after each vaccination.
- Number of participants who experienced serious adverse reactions/events during the whole period of observation (0-7 months).

Statistical Analysis Plan

Immunogenicity analysis:

The per-protocol population who met the following criteria was involved in the immunogenicity analysis: 1) satisfied the inclusion criteria for the first dose; 2) without the exclusion condition for the 1st, 2nd and 3rd doses; 3) completed three doses of Hecolin® and blood sample collection at month 7. The immune response was defined as achieving positive anti-HEV IgG at month 7, which was summarized as positive rate and geometric mean concentration (GMC) with 95% confidential intervals (CI) respectively. The difference value of positive rate and GMC ratio of anti-HEV IgG between the CHB group and the control group was calculated with 95%CI. The criteria of the immunogenicity non-inferiority between CHB group and healthy adults group were: 1) the lower limit of 95% CI of the seroconversion rate difference was not less than -10%, and 2) the lower limit of 95%CI of the GMC ratio was not lower than 0.5.

Safety analysis:

The participants who had completed at least one dose of study vaccine and the safety record after the dose were involved in the safety analysis and the participants who went against the protocol after the first dose were not excluded. The reporting rate of AE was calculated and compared by Pearson χ^2 test or Fisher's exact test between the two groups. The effect of hepatitis E vaccination on the liver function was estimated for three times by comparing the value of AST, ALT and TBIL before and one month after the first dose, before and one month after the third dose, before the first dose and one month after the third dose respectively. Changes in the blood test parameters of

ALT, AST and TBIL for each participant were determined by comparing the results of paired blood samples. The fluctuations were classified into three categories: “no change” indicated no grade change; “processed” indicated a change from normal to abnormal or an increase in grade; and “improved” indicated a change from abnormal to normal or a decrease in grade or the change from abnormal to normal. The grade of ALT, AST and TBIL was determined according to the rules issued by China Food and Drug Administration (CFDA). The range of normal value of ALT, AST and TBIL was 0-50 U/L, 0-40 U/L and 2.0-20.4 $\mu\text{mol/L}$ respectively for males, and was 0-40 U/L, 0-35 U/L and 2.0-20.4 $\mu\text{mol/L}$ respectively for females.

The data analyses were performed using R software. $P < 0.05$ was considered to be statistically significant.