Immunization against Hepatitis E

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Soon after the 1991 molecular cloning of hepatitis E virus (HEV), recombinant viral capsid antigens were expressed and tested in nonhuman primates for protection against liver disease and infection. Two genotype 1 subunit vaccine candidates entered clinical development: a 56 kDa vaccine expressed in insect cells and HEV 239 vaccine expressed in *Escherichia coli*. Both were highly protective against hepatitis E and acceptably safe. The HEV 239 vaccine was approved in China in 2011, but it is not yet prequalified by the World Health Organization, a necessary step for introduction into those low- and middle-income countries where the disease burden is highest. Nevertheless, the stage is set for the final act in the hepatitis E vaccine story—policymaking, advocacy, and pilot introduction of vaccine in at-risk populations, in which it is expected to be cost-effective.

Identification of a novel virus pathogen that is geographically dispersed, transmissible by the fecal–oral route, responsible for massive disease outbreaks, and capable of producing serious and life-threatening illness in vulnerable populations invariably stimulates an effort to develop prevention and control measures—first and foremost being a prophylactic vaccine. Hepatitis E virus (HEV) is such a pathogen. This review describes the 35-year history of hepatitis E vaccine development from 1983 to 2017.

Our account begins with the identification of an infectious virus-like particle (VLP) associated with enterically transmitted non-A, non-B hepatitis that was unable to be propagated in cultured cells. As molecular virology advanced, the HEV RNA genome was cloned and sequenced. This enabled development of diagnostic methods to define its epidemiology. Two hepatitis E vaccine candidates were invented,

and each produced using a different platform for recombinant expression of a fragment of the HEV capsid protein. These vaccine candidates were shown to be highly effective with acceptable safety; one is now licensed for use in China. Nevertheless, vaccine prevention and control of hepatitis E remains elusive, awaiting generation of additional evidence that immunization programs in at-risk populations are suitable and cost-effective. We close with a discussion of the information gaps that exist and steps that might be taken to make vaccination against hepatitis E available to those who need it.

IMMUNITY TO HEV

Early studies of HEV infection and disease in humans and animal models were hampered by the lack of reference reagents and the necessity

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to use immune electron microscopy to detect virus or anti-HEV. Nevertheless, by 1988, Bradley and colleagues at the U.S. Centers for Disease Control and Prevention (CDC) reported immune electron microscopy data (Bradley et al. 1988), showing that one antigenically related class of viruses, visualized as antibody-coated 32-nm VLPs, is responsible for the majority of human disease that was at the time termed enterically transmitted non-A, non-B hepatitis. They used human stool suspensions containing abundant 32-nm VLPs that were collected from a patient with acute hepatitis in Tashkent, Uzbekistan and a patient in Telixtac, Mexico to show that acute illness serum specimens from numerous other patients with well-documented non-A, non-B hepatitis in the USSR, Pakistan, Nepal, Burma, Sudan, Somalia, and Mexico contained immunoglobulins that bound to these VLPs.

Following the determination of the first complete RNA sequence of the HEV genome (Tam et al. 1991), the complete RNA sequence of another HEV isolate (SAR-55) from a hepatitis E outbreak in a secondary school located in Sargodha, Pakistan was determined (Tsarev et al. 1992) and used to produce recombinant HEV antigens using a baculovirus expression system. These enabled serologic tests for anti-HEV to be developed (Tsarev et al. 1993) and set the stage for a burst of epidemiologic field studies to characterize humoral immunity to HEV and preclinical studies to evaluate recombinant hepatitis E vaccine candidates.

ANIMAL MODELS TO EVALUATE IMMUNITY

Transmission of HEV from an infected human volunteer to cynomolgus monkeys by intravenous inoculation of a virus-containing stool extract was first reported in 1983 (Balayan et al. 1983). This landmark report presented evidence that 27- to 32-nm VLPs visualized by immune electron microscopy in a pool of stool extracts from patients with presumed acute enterically transmitted non-A, non-B viral hepatitis caused acute hepatitis after an incubation period of ~35 days when administered orally to an adult volunteer. A stool filtrate from that volunteer

collected 42 days postinoculation also contained antigenically similar VLPs. This filtrate caused acute hepatitis when administered intravenously to two cynomolgus monkeys; similar VLPs were observed in the monkeys' stool specimens beginning 10 and 16 days after inoculation. Transmission of acute hepatitis after an incubation period of several weeks by intravenous inoculation of nonhuman primates with VLP-containing stool filtrates was replicated repeatedly thereafter (Kane et al. 1984; Andjaparidze et al. 1986; Bradley et al. 1987; Arankalle et al. 1988). The development of this nonhuman primate model of disease resulted in the generation of virus-containing materials (feces, bile, and liver) that enabled isolation of the first complementary DNA (cDNA) clone coding for a portion of the HEV RNA genome (Reyes et al. 1990).

The first report of a hepatitis E vaccine candidate was published in 1993 by scientists at the CDC who assessed protection of cynomolgus macaques elicited by trpE-C2 protein, a trpE-HEV fusion protein that represented the carboxyl two-thirds of the open reading frame 2 capsid protein (pORF2) from a genotype (gt)1 HEV isolate (Burma) expressed in *Escherichia coli* (Purdy et al. 1993). Two animals immunized thrice with the candidate were protected from biochemical and histopathologic hepatitis when challenged with HEV (Burma) or HEV (Mexico). This pilot study was notable, as it offered the first evidence that vaccine prevention of hepatitis E may be feasible.

An important advance came when investigators at the U.S. National Institutes of Health (NIH) titrated the infectivity of the SAR-55 strain of HEV in cynomolgus macaques (Tsarev et al. 1994a), thereby generating a virus stock enabling reproducible challenge experiments necessary for testing the efficacy of vaccine candidates. This group then produced a recombinant baculovirus-expressed pORF2 polypeptide (initially termed a 55 kDa polypeptide, but subsequently characterized as a 56 kDa species), purified it chromatographically, and adsorbed it to aluminum hydroxide (AlOH) as a hepatitis E vaccine candidate. Cynomolgus monkeys were then administered saline placebo or the vaccine

candidate (one or two 50- μ g doses 4 weeks apart) and challenged intravenously with SAR-55 HEV 4 weeks after immunization. All control animals developed histopathologically confirmed hepatitis with HEV RNA detected in serum (viremia) and feces; all vaccinated animals developed anti-HEV (reciprocal titers at the time of challenge ranged from 100 to 10,000) and were protected from hepatitis and viremia, although animals receiving only a single vaccine dose were infected as evidenced by detection of HEV in feces, whereas animals receiving two vaccine doses were not (Tsarev et al. 1994b). The anti-HEV was optimally detected by an enzyme-linked immunosorbent assay (ELISA) using vaccine-homologous antigen. As part of the same challenge experiment, four additional animals received late convalescent plasma from a cynomolgus monkey that had been infected experimentally with a Chinese isolate of HEV, having the same capsid protein amino acid sequence as SAR-55. These animals had anti-HEV reciprocal titers of 40–200 when challenged, but these levels decayed during the days immediately after virus challenge. Three of four animals were protected from histopathologic signs of hepatitis, but all four animals were infected as manifested by serum viremia and multiweek excretion of HEV in feces.

PRECLINICAL EVIDENCE THAT IMMUNITY TO A 56 kDa RECOMBINANT HEPATITIS E VACCINE CANDIDATE CONFERS HETEROLOGOUS PROTECTION

By 2001, an analysis of full length genomic sequences for HEV isolates collected from humans and animals on several continents clarified that HEV has evolved into at least four antigenically related genotypes (Schlauder and Mushahwar 2001): gt1 is represented by patient isolates from Asia and North Africa, gt2 by patient isolates from Mexico and West Africa, gt3 by patient and swine isolates from the United States and Europe, and gt4 by patient and swine isolates from China (see Smith and Simmonds 2018). Initial vaccine discovery efforts assessed candidate vaccines composed of polypeptides representing portions of pORF2 from

gt1 HEV isolates, as this genotype appears to be responsible for the largest amount of human disease.

The CDC vaccine candidate based on the gt1 Burma HEV isolate discussed above (Purdy et al. 1993) was not evaluated further, and was quickly supplanted by the NIH's hepatitis E vaccine development program, which reported protection data in 1994 (Tsarev et al. 1994a). The NIH candidate was a recombinant capsid polypeptide from a different gt1 HEV (SAR-55, Pakistan) expressed in Sf9 cells of the fall armyworm, *Spodoptera frugiperda*, using a baculovirus vector (*Autographa californica* nuclear polyhedrosis virus). The production and purification process for this 56 kDa HEV capsid protein vaccine was further developed (Robinson et al. 1998) and, by 1998, a vaccine lot suitable for investigational use in humans was manufactured. To evaluate the breadth of protection afforded by the 56 kDa HEV vaccine, immunized rhesus monkeys were challenged via the intravenous route 8 weeks after vaccination with 10,000 50% monkey-infective doses with SAR-55 (gt1, vaccine homologous strain), Mex-14 (gt2, vaccine heterologous strain), and US-2 (gt3, vaccine heterologous strain). Two-dose vaccination with either 1- or 10- μ g doses afforded 100% protection relative to control animals (immunized with hepatitis A vaccine) against hepatitis (monitored by alanine aminotransferase levels in serum) and 63%–84% protection against infection manifest as serum viremia (Purcell et al. 2003). The level of serum anti-HEV immunoglobulin (Ig)G by ELISA using vaccine homologous antigen (SAR-55) was quantified in specimens collected before virus challenge. Results were determined in World Health Organization (WHO) units/mL, based on use of the WHO Reference Reagent for HEV (The National Institute for Biological Standards and Control, NIBSC code 95/584; see www.nibsc.org/documents/ifu/95-584.pdf). Fully protected animals (two-dose recipients) had a geometric mean concentration (GMC) of anti-HEV IgG of 483 WHO U/mL; partially protected animals (one-dose recipients) had a GMC of 61 WHO U/mL. These results provided important pre-clinical evidence that a gt1 recombinant hepati-

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tis E vaccine could broadly protect humans against hepatitis E.

NEUTRALIZING EPITOPES IN THE HEV CAPSID PROTEIN

An important function of the humoral immune response to viruses is virus neutralization. Detection of HEV replication in a human hepatocellular carcinoma cell line (PLC/PRF/5) over 3 weeks by polymerase chain reaction (PCR) created a means to detect virus neutralization in vitro (Meng et al. 1997). Using this test, antibodies against an HEV recombinant “C2” protein comprising the carboxy-terminal two-thirds (225–660 aa) of the HEV Burma strain pORF2 capsid protein were shown to neutralize HEV strains from Burma, Mexico, and Pakistan (Meng et al. 1998). Subsequently, the location of the neutralizing epitope in the capsid protein was mapped using overlapping 30-mer synthetic peptides spanning the entire C2 protein and 31 overlapping recombinant proteins of different sizes derived from the entire Burma pORF2 to immunize mice (Meng et al. 2001). Immune sera were tested by the in vitro neutralization assay. Antibodies against synthetic peptides and recombinant polypeptides <100 amino acids in length had no neutralizing activity, suggesting that the HEV neutralizing epitope is conformational rather than linear. In line with this observation, antibodies against recombinant proteins containing amino acids 452–617 showed cross-reactive HEV neutralizing activity, supporting the concept that the neutralizing epitope resides within this carboxyl-terminal portion of the capsid protein. This work was extended when two monoclonal antibodies that neutralized HEV were used to identify a subregion of the ORF2 capsid protein spanning amino acids 459–607 as the shortest peptide to form the corresponding neutralization epitopes (Zhou et al. 2004). An ELISA that was based on a recombinant protein spanning amino acids 458–607 in ORF2 of the SAR-55 strain (gt1) was efficient in detecting anti-HEV in nonhuman primates that had been experimentally infected with HEV gt1, gt2, gt3, or gt4 (Zhou et al. 2004). These data indicate that HEV exists as a

single serotype despite the characterization of four phylogenetically distinct genotypes, and imply that immunity elicited by a vaccine containing a fragment of the gt1 ORF2 capsid protein comprising the carboxyl-terminal neutralization epitope will be cross-protective.

IMMUNITY TO HEV IN HUMANS

The development of an HEV ELISA (SAR-55 strain) enabled sero-epidemiology studies to be conducted, including a retrospective study of the outbreak in Pakistan that yielded the SAR-55 strain (Bryan et al. 1994). There were two critical observations in that outbreak study. The first was that hepatitis E in young adults is the outcome of a primary HEV infection, as anti-HEV IgM was detected in virtually every case. More importantly, the presence of anti-HEV IgG was correlated with resistance to hepatitis E. Among contacts of patients with hepatitis E, 33% of those with neither anti-HEV IgM nor IgG when initially studied were later hospitalized with hepatitis E. In contrast, no individuals with anti-HEV IgG but without IgM were later hospitalized.

An ELISA to quantitate total Ig to HEV, using the 56 kDa capsid protein expressed by recombinant baculovirus in insect cells as the immunoassay antigen, was validated in anticipation of its use to assess the immunogenicity of recombinant hepatitis E vaccine in clinical trials (Innis et al. 2002). The test was used to quantify the antibody responses of six healthy pregnant women in Nepal who had serum collected while healthy (preinfection), when they became ill with hepatitis E (with jaundice, elevated alanine transaminase [ALT] levels in serum, and detection of HEV RNA by reverse transcription PCR [RT-PCR] in stool or serum specimens), and during late convalescence. Antibody levels were expressed in Walter Reed (WR) U/mL; however, these units could be converted to WHO U/mL by multiplying by 0.125. Among these six women, the geometric mean antibody level pre-illness was 0.9 WHO U/mL, rose to 240 U/mL on the day of illness evaluation, and declined slightly to 90 WHO U/mL, a median of 140 days later (range, 38–275 days). The kinetics

of anti-HEV Ig in serum as determined by the same immunoassay were further characterized in a cohort of 62 adults from Nepal with acute hepatitis E (Myint et al. 2006); the median acute Ig level was 788 WHO U/mL, which declined rapidly over 3 months and then at a slower rate. Late convalescent samples from ~400 days later in 16 patients ranged from 4 to 125 WHO U/mL (median value ~31 WHO U/mL).

An ELISA to quantitate IgM to HEV using the same 56 kDa capsid protein as the immunoassay antigen was also validated in anticipation of its use to support diagnosis of acute hepatitis E in clinical trials of the vaccine (Seriwatana et al. 2002). Among 36 patients hospitalized with acute hepatitis E, the geometric mean level of anti-HEV IgM was 3000 WR U/mL a median of 8 days after symptom onset, declining to 100 WR U/mL, a median of 190 days after symptom onset. Anti-HEV IgM >100 WR U/mL was detected in serum from >95% of 197 patients with acute hepatitis E diagnosed by detection of HEV RNA in serum by RT-PCR. In this series of 197 cases, 189 were considered primary infections based on the ratio of IgM to total Ig. On the other hand, among the eight cases with low IgM-to-Ig ratios, the levels of IgM were low and the levels of total Ig were extremely high. These findings extended the results of Bryan et al. (1994) and strengthened the observation that typical hepatitis E occurs following a primary HEV infection, although a small fraction of disease may occur following reinfection characterized by an anamnestic antibody response having a low level of IgM and a very high level of IgG.

CLINICAL DEVELOPMENT OF THE 56 kDa VACCINE CANDIDATE

The U.S. Department of Defense has considered hepatitis E to be a potential medical threat to military and peacekeeping operations in Asia and Africa since the 1980s, when it noted that military personnel of the Soviet Union sustained high rates of hepatitis during operations in Afghanistan. Accordingly, the U.S. Army Medical Research and Materiel Command (USAMRMC) sponsored research directed at

developing a hepatitis E vaccine as early as 1985. The initial aim was to develop diagnostic methods for enterically transmitted non-A, non-B hepatitis, as hepatitis E was first designated, and to describe its epidemiology. Staff from the Uniformed Services University of the Health Sciences, the Walter Reed Army Institute of Research (WRAIR), and the Army Medical College, Rawalpindi established the Pakistan-U.S. Laboratory for Sero-Epidemiology, which conducted important field studies describing outbreaks of hepatitis E among military personnel (Iqbal et al. 1989; Ticehurst et al. 1992; Bryan et al. 1994, 2002). Biological specimens from this group's outbreak investigations resulted in recovery and sequence analysis of the SAR-55 strain of HEV, development of a sensitive ELISA for serology, and ultimately creation of the NIH's hepatitis E vaccine candidate.

At the same time, the WRAIR was supporting field studies of non-A, non-B hepatitis in Kathmandu, Nepal, prompted by an outbreak investigation of widespread epidemic hepatitis in 1981–1982, conducted by the CDC and the Ministry of Health, Nepal (Kane et al. 1984). A notable feature of that epidemic was the description of a 21% case fatality ratio in affected pregnant women. Subsequent work with staff from the Teku Infectious Diseases Hospital (Kathmandu) of the Ministry of Health and the Nepalese Army Medical Department documented the importance of hepatitis E as a common cause of serious disease among civilian and military personnel (Clayson et al. 1995, 1997, 1998). Sequence analysis of six stool specimens collected from patients hospitalized with hepatitis E in Kathmandu between 1987 and 1995 showed that all were closely related to contemporary isolates of HEV from Burma and India, that is, gt1 (Gouvea et al. 1997).

When the possibility of vaccinating humans against hepatitis E was suggested by nonhuman primate studies conducted by the NIH, the USAMRMC and WRAIR entered into a collaboration with the NIH and GlaxoSmithKline (GSK) to evaluate the feasibility of developing a vaccine against hepatitis E. This collaboration built on the relationships among these same partners, which resulted in GSK developing

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the first vaccine to be licensed for prevention of hepatitis A (Hoke et al. 1992).

The hepatitis E vaccine candidate developed by the NIH that was evaluated by the USAMRMC was the same purified 56 kDa product, expressed by a recombinant baculovirus in Sf9 cells and adsorbed to 0.5 mg of aluminum as ALOH per 0.5 mL dose, which Purcell and colleagues used for preclinical tests of safety and efficacy. Investigators at the WRAIR conducted a phase I randomized, open-label escalating dose trial in 88 healthy HEV-seronegative U.S. adults 18–50 years of age to evaluate the safety and immunogenicity of a range of vaccine doses (1, 5, 20, or 40 µg of capsid protein) administered three times on a 0-, 1-, and 6-month schedule. All dose levels had an acceptable safety profile; the three highest dose levels elicited an anti-HEV Ig response 1 month after dose 3 to at least 5 WHO U/mL in ≥88.9% of subjects with GMCs of 24, 38, and 31 WHO U/mL, respectively (Safary 2001). Investigators at the Walter Reed-Armed Forces Research Institute of Medical Sciences Research Unit Nepal then conducted a phase II dose confirmation trial of the vaccine candidate in healthy adults in Kathmandu. Forty-four seronegative subjects were randomly allocated (1:1) to dose levels of 5 and 20 µg administered on the same three-dose schedule. One-month post-dose 3, the anti-HEV Ig seropositivity (i.e., ≥2.5 WHO U/mL) rates and GMCs were 94% and 17 WHO U/mL (5 µg group) versus 100% and 47 WHO U/mL (20 µg group) (BL Innis, pers. comm.). The safety profile in both groups was acceptable. Based on these two studies, the 20-µg dose was selected for a phase II proof-of-concept study to assess the vaccine's protective efficacy.

As the proof-of-concept trial was designed to provide a preliminary assessment of safety and efficacy with a limited sample size, the investigators elected to screen volunteers for antibody to HEV by ELISA so that only susceptible individuals would be randomized to vaccine or placebo (Shrestha et al. 2007). Plans to conduct the trial in the city of Lalitpur, adjacent to Kathmandu, were set aside when local government officials objected (Stevenson 2000). As the burden of hepatitis E among military personnel was

similar to that among civilians, the Nepalese Army, with the concurrence of the Ministry of Health, agreed to host the study among soldiers based in the Kathmandu Valley, a decision that generated some controversy in retrospect despite ethical review of the proposed study in both the United States and Nepal (Bhattarai 2007). Nevertheless, the double-blind trial was initiated in 2001, with more than 40,000 soldiers being informed about the study, 5323 consenting to screening, 3323 (62%) being considered seronegative, and 2000 soldiers (mean age 25 years, range 18–62 years, 99.6% male) consenting to be randomized 1:1 to receive three doses of vaccine or placebo.

Surveillance for acute hepatitis was conducted among 1566 subjects who were followed for a median of 804 days. The Data and Safety Monitoring Board (DSMB) reviewed 111 episodes of acute hepatitis and certified 87 as definite hepatitis E before unblinding the trial. These cases were confirmed by biochemical markers of liver disease (ALT >2.5 times upper limit of normal and/or total serum bilirubin >2.0 mg/dL), detection of HEV RNA in serum or stool, and anti-HEV IgM or total Ig in serum. The median duration of illness was 29 days; the median maximum serum alanine aminotransferase was 1248 U/L; the median maximum serum total bilirubin was 9.0 mg/dL.

The trial's primary objective was to evaluate the efficacy of a three-dose vaccination course. From 14 days after the third vaccine dose until study end, 69 subjects developed hepatitis E—three in the vaccine group (0.3%) and 66 in the placebo group (7.4%)—for a vaccine efficacy of 95.5% (95% CI, 85.6–98.6). A secondary objective was to evaluate the efficacy of a two-dose vaccination course. From 14 days after the second vaccine dose until the administration of the third dose (~5 months later), eight subjects developed hepatitis E—one in the vaccine group (0.1%) and seven in the placebo group (0.7%)—for a vaccine efficacy of 85.7% (95% CI, –16.0–98.2).

The vaccine was well tolerated with a reactogenicity profile similar to placebo, except that injection-site pain occurred more frequently among vaccine recipients. There were no clini-

cally notable differences between the vaccine and control groups with respect to spontaneously reported adverse events or serious adverse events (SAEs). Although there were six deaths in the vaccine group and only one in the placebo group, none of the deaths were considered to be related to vaccination. Given the modest size of the trial, however, the accumulated safety experience was insufficient to assess the risk of rare vaccine-related adverse events.

The vaccine was immunogenic. Among subjects in the immunogenicity subgroup who received vaccine, 81.3% had a level of anti-HEV Ig of at least 2.5 WHO U/mL 1 month after the second vaccine dose, and 100% had this level 1 month after the third vaccine dose. By study end, the proportion had declined to 56.3%. Despite the declining proportion of seropositive vaccine recipients, there was no apparent diminution of protection, consistent with the establishment of immunologic memory, recallable in time to limit infection and prevent disease during the average 5- to 6-week incubation period of hepatitis E.

The results of the proof-of-concept trial strongly supported the feasibility of preventing hepatitis E by vaccination and clearly showed the important contribution of hepatitis E to overall morbidity among the subjects in the study, as hepatitis E was the most common medically significant illness resulting in hospitalization and disability in the placebo group. The investigators concluded that vaccination against hepatitis E had high potential to improve well-being among adults with similar disease exposures.

At the conclusion of the trial, GSK sought public-sector partners who were willing to invest in further development of the 56 kDa vaccine candidate for use where hepatitis E is endemic. None were forthcoming. Moreover, public health systems in countries where hepatitis E remains endemic have made introduction of life-saving rotavirus and pneumococcal conjugate vaccines for infants a higher priority. Given these obstacles, and the emergence of a competing hepatitis E vaccine manufactured using a less costly bacterial expression system (HEV 239, described below), GSK halted development of the 56 kDa vaccine.

DISCOVERY AND PRECLINICAL DEVELOPMENT OF THE HEV 239 VACCINE CANDIDATE IN CHINA

Hepatitis E is endemic in China. In an epidemiological review from 1991, Zhuang and colleagues (Zhuang et al. 1991) reported the occurrence of nine putative hepatitis E outbreaks since 1982, the largest of which caused more than 120,000 cases between September 1986 and April 1988 in Xinjiang Uighur Autonomous Region in China's northwest. In this outbreak, there were 707 deaths with 414 occurring in pregnant women. Analysis of stool specimens from patients in this and other outbreaks confirmed the etiology as HEV belonging to gt1 (Aye et al. 1992; Yin et al. 1993). In response to the growing recognition of the hepatitis E burden in China, a research program at Xiamen University was established in 1998 to develop a diagnostic kit to facilitate diagnosis and, in parallel, a hepatitis E vaccine to prevent disease (Wu et al. 2012a).

The hepatitis E vaccine development program aimed to express a polypeptide from the HEV capsid protein in *E. coli* that contained neutralizing epitopes presented in particulate form. The full-length capsid protein can be divided into the S domain (amino acids 118–313), the P1 domain (amino acids 314–453), and the P2 domain (amino acids 456–606). The P2 domain containing 153 amino acids is the outermost moiety of the viral capsid. Termed the protrusion domain, it is essential for viral–host interaction, and contains the known neutralizing epitopes (Li et al. 2009).

To achieve their aim, the developers generated varying lengths of the capsid gene from a human isolate of a gt1 virus first isolated from the fecal extract of a patient in the Xinjiang Uighur Autonomous Region in 1988 (DNA Data Bank of Japan [DDBJ] accession no. D11092) (Aye et al. 1992). These were cloned into the pT0-T7 expression plasmid to generate recombinant proteins of different length using an expression system. These various proteins were evaluated for structure and immunoreactivity with acute patient serum. The HEV E2 capsid polypeptide, consisting of amino acids

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394–607, was an early candidate vaccine antigen as it self-assembled into dimers. This was desirable because Zhang and others, using murine monoclonal antibodies to the HEV capsid protein, showed that its major neutralizing epitopes are formed by discontinuous amino acids between positions 470 and 606, and are associated with a dimeric rather than a monomeric conformation (Zhang et al. 2005). The E2 polypeptide elicited neutralizing antibodies and protected against viral challenge when administered to macaques with Freund's adjuvant. However, E2 without Freund's adjuvant was poorly immunogenic in mice and monkeys (Li et al. 2005b).

Consequently, the developers systematically evaluated the role of the amino acids between the amino terminus and carboxyl terminus in dimer and particle formation. Although the minimal dimerization domain was located at amino acids 459–601, further extension of the amino terminus to amino acid 368 was necessary for particle formation (Li et al. 2005a; Wei et al. 2014). Thus, a second candidate antigen, HEV 239 (amino acids 368–606) that had an amino-terminal extension relative to E2, was evaluated and found to form dimers that self-assemble into VLPs. Biophysical analysis of the HEV 239 VLPs by high-performance size-exclusion chromatography, analytical centrifugation, and electron microscopy clearly showed particles with diameters of 20–30 nm (Li et al. 2005b). HEV 239 was selected as the vaccine antigen because it showed 200-fold enhancement in immunogenicity in mouse and primate experiments compared with E2 (Li et al. 2005b; Wu et al. 2007). HEV 239 VLPs present neutralizing epitopes as confirmed by binding studies using well-characterized neutralizing murine monoclonal antibodies 8C11 (specific for gt1 and gt2) and 8G12 (cross-genotype reactive) (Wei et al. 2014; Zhao et al. 2015). HEV 239 VLPs adsorbed to AIOH were administered as two doses 4 weeks apart at either 5, 10, or 20 μ g per dose and completely protected rhesus macaques from hepatitis and infection when challenged at week 7 with 10^4 genome copies (100 infective doses) of homologous gt1 or heterologous gt4 HEV. At a higher challenge dose of 10^5 infective doses, all dosing regimens completely

protected against hepatitis and partially protected against infection (Li et al. 2005b).

CLINICAL DEVELOPMENT OF THE HEV 239 VACCINE CANDIDATE PRELICENSURE

The HEV 239 vaccine candidate evaluated in a phase I study in 44 healthy HEV seronegative adults contained 20 μ g HEV 239 adsorbed to AIOH. When administered twice by the intramuscular route at a 1-month interval, it had an acceptable reactogenicity and safety profile. Subsequently, a phase II clinical trial was conducted in two parts to assess schedule and dose escalation. Part A enrolled 457 adults, all seronegative for HEV, who received 20 μ g of HEV 239 twice (months 0 and 6) or thrice (months 0, 1, and 6). Subjects in the three-dose group had similar seroconversion rates to those in the two-dose group (100% vs. 98%), but a higher GMC of anti-HEV IgG (15.9 vs. 8.6 WHO U/mL (Zhang et al. 2009). Part B was a dose-finding study in 155 high school students at least 16 years of age, each of whom received three doses of HEV 239 (0–1–6) at one of four different dose levels (10, 20, 30, or 40 μ g). All four groups had 100% seroconversion with the three higher dose groups having better anti-HEV GMCs than the 10- μ g dose group. Adverse event profiles were similar across all groups (Zhang et al. 2009). Based on these results, a three-dose regimen of 30 μ g of HEV 239 adsorbed to 0.8 mg AIOH and suspended in 0.5 mL of buffered saline administered intramuscularly at months 0, 1, and 6 was advanced to phase III.

The safety and efficacy of HEV 239 vaccine was evaluated in a randomized, double blind, placebo-controlled, single-center phase III clinical trial conducted in a known endemic location, Dongtai, Jiangsu Province, China from August 2007 to May 2009 (Zhu et al. 2010). A total of 112,604 subjects 16–65 years of age were randomly assigned (1:1) to receive HEV 239 or a licensed hepatitis B control vaccine intramuscularly at 0, 1, and 6 months. Hepatitis E cases were identified by active surveillance performed at 205 sentinel sites. The hepatitis E case definition included (1) clinical symptoms of acute hepatitis illness lasting for at least 3 days, (2) diagnosis of

acute hepatitis by ALT ≥ 2.5 times the upper limit of normal, and (3) at least two of three positive HEV markers (anti-HEV IgM, HEV RNA, or a fourfold or greater increase in anti-HEV IgG). Safety and efficacy were first assessed and reported at 1 year (beginning 30 days after the final dose), and again after an extended follow-up analysis at 55 months.

The trial's primary objective was to evaluate prevention of hepatitis E during the first year of follow-up beginning 30 days after the last dose (Table 1). The DSMB certified 23 cases of hepatitis E before unblinding for the primary analysis. Among cases, the mean maximum serum ALT was 30.8 times upper limit of normal and the mean duration of illness was 57.1 days with 15 patients admitted to a hospital. Per-protocol vaccine efficacy was 100% (95% CI, 72.1–100) with no cases of hepatitis E among vaccine recipients and 15 cases among placebo recipients. In an intention-to-treat analysis considering all who received at least one dose, there was one case of hepatitis E among vaccine recipients (this participant had received only one dose). All remaining 22 cases were in the placebo group, resulting in a vaccine efficacy of 95.5% (95% CI, 66.3–99.4) (Zhu et al. 2010). The efficacy after administration of two doses measured during the 5-month interval between dose 2 and 3 was also 100% (95% CI, 9.1–100), which suggests that two doses administered 1 month apart in an emergency or outbreak setting might offer protection for at least 5 months. Of note, the Chinese State Food and Drug Administration (SFDA) in their review of the HEV 239 vaccine's license application applied a less stringent criterion for biochemical evidence of liver injury to redefine cases of hepatitis E, that is, ALT greater than the upper limit of normal. Whereas this cutoff is more sensitive for detecting mild cases of illness associated with HEV infection, it likely misclassified mild illness in both groups as medically important hepatitis E and biased the efficacy estimate downward. With the less stringent case definition, the analysis identified nine cases of hepatitis E in the vaccine group and 26 in the control (as compared with 0 and 15 cases in the per-protocol analysis) with 48,500 person-years observed in each group. The resulting vaccine efficacy esti-

mates reported in the approved product insert are 65% (95% CI, 26–84) for the per-protocol analysis and 67% (95% CI, 38–82) for an intention-to-treat analysis. They should be viewed as conservative estimates of vaccine benefit.

In the extension of the phase III clinical trial, the blind was maintained and all subjects were followed out for 4.5 years (55 months) using the same surveillance system. The efficacy of HEV 239 vaccine against clinically apparent hepatitis E disease according to the protocol case definition (ALT > 2.5 times upper limit of normal) was 93.3% (95% CI, 78.6–97.9) in a per-protocol analysis and 85.1% (95% CI, 67.1–93.3) in an intention-to-treat analysis (Zhang et al. 2015). An analysis of follow-up at 7 years postvaccination is ongoing (W Huang, pers. comm.).

As was expected in the geographic area of the clinical trial, 26 of 29 HEV isolates obtained from subjects were gt4; the others were gt1. The high efficacy afforded by the gt1 HEV 239 vaccine against disease caused by gt4 HEV supports the concept that the diverse HEV genotypes form a single serotype and HEV 239 vaccine can protect against all genotypes. Further supporting this concept are immunogenicity data generated with a monoclonal antibody competition assay using the broadly cross-neutralizing murine monoclonal 8G12, which detected broadly cross-neutralizing antibody in serum specimens from HEV 239 vaccine recipients (Wu et al. 2017).

Prevention of subclinical infection by administration of HEV 239 vaccine was first shown in the phase II study and confirmed in the phase III immunogenicity cohort (Zhang et al. 2009, 2014). In both studies, subclinical infections were identified by comparing paired serum samples collected at intervals starting 30 days after the last dose. A subclinical infection was defined by seroconversion (from negative to 0.154 WHO U/mL twice the test cutoff level), or a fourfold or greater increase in titer. In the phase II study, two doses of 20 μ g HEV 239 administered 1 month apart afforded efficacy against subclinical infection of 86% (95% CI, 18–99) over a 4-month period (Zhang et al. 2009). In the phase III trial, the efficacy against subclinical infection over a 24-month period

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Table 1. Efficacy of the hepatitis E virus (HEV) 239 vaccine against hepatitis E in the phase III trial

	Follow-up (month of study)	Vaccine group			Placebo group			p Value
		Number of participants/ person-years at risk	Incidence (per 10,000 person- years)	Number of participants/ person-years at risk	Incidence (per 10,000 person- years)	Number of cases	Vaccine efficacy (95% CI)	
Per-protocol								
Whole group (three doses)	7–19	48,693/48,594.6	0.0	48,663/48,555.1	3.1	15	100.0% (72.1–100.0)	<0.0001
Men	7–19	20,662/20,616.1	0.0	20,709/20,660.0	5.3	11	100.0% (60.1–100.0)	0.001
Women	7–19	28,031/27,978.5	0.0	27,954/27,895.1	1.4	4	100.0% (–51.0–100.0)	0.045
Age 16–49 years	7–19	30,374/30,299.5	0.0	30,355/30,276.9	2.0	6	100.0% (15.13–100.0)	0.014
Age 50–65 years	7–19	18,319/18,295.2	0.0	18,308/18,278.2	4.9	9	100.0% (49.4–100.0)	0.003
First 6 months of follow-up	7–13	48,693/23,981.9	0.0	48,663/23,965.8	2.5	6	100.0% (15.12–100.0)	0.014
Second 6 months of follow-up	14–19	48,693/24,612.8	0.0	48,663/24,589.3	3.7	9	100.0% (49.4–100.0)	0.003
First two doses subset	1.5–5	54,986/20,202.1	0.0	54,973/20,196.8	2.5	5	100.0% (9.1–100.0)	<0.0001
Population receiving at least one dose	7–19	56,302/56,104.7	0.2	56,302/56,081.2	2.9	16	93.8% (59.8–99.9)	0.0001
Modified subset two (participants received at least one dose)	0–19	56,302/87,354.2	0.1	56,302/87,323.2	2.5	22	95.5% (66.3–99.4)	<0.0001
Modified subset two (participants in reactogenicity subset were excluded because of lacking follow-up data during 0–6 months)	0–19	54,986/86,040.4	0.1	54,973/86,003.4	2.4	21	95.2% (64.6–99.4)	<0.0001

Person-years at risk is the cumulative follow-up years of at-risk participants at the indicated time point. Number of at-risk participants is the initial number of participants entered in the study (cumulative hepatitis E cases + participants who had dropped out of the study). (From Zhu et al. 2010; reprinted, with permission, from Elsevier © 2010.)

postvaccination was 78% (95% CI, 65–86) in a per-protocol analysis and 77% (95% CI, 65–85) in an intention-to-treat analysis.

Reactogenicity data in the phase III trial was solicited from a subset of participants (~2600) from one township; these subjects were intensively followed for solicited and spontaneously reported adverse events (Table 2). Most adverse events were mild. Although the frequency of solicited injection site adverse events was higher in the vaccine group than the hepatitis B vaccine control group (2.8% vs. 1.9%, perhaps because of the 30 µg versus 5 µg disparity in antigen content), the frequency of solicited systemic adverse events was similar between the two groups. Additionally, adverse events in the phase III trial were collected from the total vaccinated cohort (Table 2). No safety signal was detected during the first 19 months of the trial. Over the extended 4.5-year follow-up period, a similar number of participants reported SAEs in the vaccine and placebo groups. None of the SAEs were attributed to the investigational vaccine. Overall, HEV 239 was considered to have an acceptable safety profile.

Although pregnancy was an exclusion criterion for enrollment, there were 37 women in the vaccine group and 31 in the control group who were inadvertently administered vaccine during pregnancy (Wu et al. 2012b). The rates of adverse events were similar between the women in the HEV 239 group and the control vaccine recipients, as were the anthropometrics and gestational ages of the infants.

More than 11,000 vaccine and control recipients were included in an immunogenicity subset and had serum samples taken before vaccination and 1 month following the third dose. A complete three-dose course of HEV 239 elicited antibody responses in 99.9% of those seronegative at baseline with peak GMC of antibody of 15 WHO U/mL 1 month after the last vaccination. This titer is considerably higher than found in persons after they recover from natural infection (0.6 WHO U/mL) but is lower than that elicited by acute hepatitis E (80.9 WHO U/mL) (Zhang et al. 2014).

At 55 months after first vaccination, 87% percent of these subjects remained seropositive; however, the GMC had declined to 0.27 WHO

U/mL. Despite this antibody decline, vaccine efficacy remained high at 93% (95% CI, 79–98). The antibody levels of subjects seronegative at baseline who received only two doses of HEV 239 were only slightly lower than the levels induced by three doses at 55 months, suggesting that a two-dose regimen might be a feasible future vaccination strategy. A modeling analysis to estimate the long-term persistence of antibody was conducted using a subpopulation from the per-protocol immunogenicity cohort that had serum samples collected at multiple time points out to 5 years from one township, followed by a similar validation subpopulation from a second township to assess the robustness of the model. A well-fitted modified power-law model, which accounts for both activated and memory B-cell decay, predicted that half of the baseline seronegative subjects would have detectable antibody titers for more than 30 years (Andraud et al. 2012; Chen et al. 2015). Among those seropositive at baseline, antibody levels were indistinguishable at 55 months regardless of whether the subject received one, two, or three doses of HEV 239.

Although there were seven breakthrough cases of hepatitis among vaccine recipients over the course of the 55-month follow-up, none of them occurred in the immunogenicity cohort; therefore, a protective antibody level could not be estimated within this trial (Zhang et al. 2015). Using the immunogenicity cohort of the phase III trial, Zhang and colleagues looked at the relative risk of 2-year cumulative infection rates among subjects, irrespective of whether they had vaccine or naturally induced antibody, against different HEV antibody levels at 1-month post-last dose. They found that those with an anti-HEV IgG level >1.0 WHO U/mL were significantly more protected against infection (Zhang et al. 2014).

REGULATORY APPROVAL AND FURTHER CLINICAL DEVELOPMENT OF THE HEV 239 VACCINE

Data from the phase III trial were submitted to the SFDA in late 2009 by the manufacturer Xiamen Innovax Biotech in Xiamen, China. The

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Table 2. Adverse events reported in the phase III clinical trial of the hepatitis E virus 239 vaccine

	Number of adverse events (rate, 95% CI)		
	Vaccine group	Placebo group	<i>p</i> Value ^a
<i>Reactogenicity subset</i>			
Number of participants who received more than one dose	1316	1329	
Solicited local adverse events within 72 h after each dose			
Local adverse events	177 (13.5%, 11.65–15.41)	94 (7.1%, 5.75–8.59)	<0.0001
Local adverse events ≥ grade 3	2 (0.2%, 0.02–0.55)	0 (0.0%, 0.00–0.28)	0.248
Pain	136 (10.3%, 8.74–12.11)	73 (5.5%, 4.33–6.86)	<0.0001
Pain ≥ grade 3	0 (0.0%, 0.00–0.28)	0 (0.0%, 0.00–0.28)	-
Swelling	30 (2.3%, 1.54–3.24)	8 (0.6%, 0.26–1.18)	<0.0001
Swelling ≥ grade 3	2 (0.2%, 0.02–0.55)	1 (0.0%, 0.00–0.28)	0.248
Itch	20 (1.5%, 0.93–2.34)	13 (1.0%, 0.52–1.67)	0.210
Itch ≥ grade 3	0 (0.0%, 0.00–0.28)	0 (0.0%, 0.00–0.28)	-
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	267 (20.3%, 18.15–22.56)	263 (19.8%, 17.68–22.03)	0.748
Systemic adverse events ≥ grade 3	7 (0.5%, 0.21–1.09)	4 (0.3%, 0.08–0.77)	0.356
Fever	245 (18.6%, 16.55–20.83)	239 (18%, 15.95–20.16)	0.674
Fever ≥ grade 3	6 (0.5%, 0.17–0.99)	3 (0.2%, 0.05–0.66)	0.341
Headache	14 (1.1%, 0.58–1.78)	8 (0.6%, 0.26–1.18)	0.191
Headache ≥ grade 3	1 (0.1%, 0.00–0.42)	0 (0.0%, 0.00–0.28)	0.498
Fatigue	28 (2.1%, 1.42–3.06)	20 (1.5%, 0.92–2.31)	0.230
Fatigue ≥ grade 3	1 (0.1%, 0.00–0.42)	0 (0.0%, 0.00–0.28)	0.498
<i>Total vaccinated cohort minus the reactogenicity subset</i>			
Number of participants who received more than one dose	54,986	54,973	
Solicited local adverse events within 72 h after each dose			
Local adverse events	1532 (2.8%, 2.65–2.93)	1051 (1.9%, 1.8–2.03)	<0.0001
Local adverse events ≥ grade 3	61 (0.1%, 0.08–0.14)	27 (0.1%, 0.03–0.07)	<0.0001
Pain	1143 (2.1%, 1.96–2.20)	754 (1.4%, 1.28–1.47)	<0.0001
Pain ≥ grade 3	1 (0.0%, 0.00–0.01)	0 (0.0%, 0.00–0.01)	1.000
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	1068 (1.9%, 1.83–2.06)	1045 (1.9%, 1.79–2.02)	0.617
Systemic adverse events ≥ grade 3	60 (0.1%, 0.08–0.14)	63 (0.1%, 0.09–0.15)	0.786
<i>Total vaccinated cohort</i>			
Number of participants who received more than one dose	56,302	56,302	

Continued

Table 2. *Continued*

	Number of adverse events (rate, 95% CI)		<i>p</i> Value ^a
	Vaccine group	Placebo group	
Unsolicited events within 30 days after each dose ^b			
All	6771 (12.0%, 11.76–12.3)	6724 (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
Serious adverse events within 30 days after each dose ^c			
All	248 (0.4%, 0.39–0.50)	245 (0.4%, 0.38–0.49)	0.892
Admission to hospital	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 ^d	10 (0.0%, 0.01–0.03)	12 (0.0%, 0.01–0.04)	0.670
Serious adverse events during period from month 2 to month 6 and from month 7 to month 19 ^b			
All	1423 (2.5%, 2.40–2.66)	1430 (2.5%, 2.41–2.67)	0.894
Admission to hospital	1328 (2.4%, 2.23–2.49)	1336 (2.4%, 2.25–2.50)	0.875
Disability	0 (0.0%, 0.00–0.01)	0 (0.0%, 0.00–0.01)	-
Death ^d	95 (0.2%, 0.14–0.21)	94 (0.2%, 0.13–0.20)	0.942

Grade 3 pain, headache, and fatigue were defined as prevention of normal activities; grade 3 swelling was defined as a diameter of more than 30 mm; grade 3 itch was defined as body itch; and grade 3 fever was defined as temperature greater than 39.0°C. Symptoms with frequency more than 1% in any group are listed.

^a*p* Values are two-sided and were calculated by Fisher's exact test.

^bUnsolicited adverse events included any adverse events that happened from day 4 to day 30 after each dose and any adverse events within 3 days after each dose, but had not been listed in the diary card for registering solicited adverse events. Most often, unsolicited adverse events in the study included upper respiratory tract infection, headache, fever, and gastritis.

^cThe Data and Safety Monitoring Board (DSMB) did not deem any of the serious adverse events to be related to vaccination.

^dTwenty-two participants died within 30 days after each vaccination. Of the 10 participants in the vaccine group that died, eight died as the result of an accident, one died of a cerebral hemorrhage, and one died of liver cancer after 10 years with chronic hepatitis B. Of 12 participants in the placebo group that died, six died as the result of an accident, three died of myocardial infarction, two died of cerebral hemorrhage, and one died of stomach cancer. (From Zhu et al. 2010; reprinted, with permission, from Elsevier © 2010.)

HEV 239 vaccine, named Hecolin (Hepatitis E vaccine, *E. coli*), was approved for use in persons 16 years of age and older in December 2011. From isolation of the parent gt1 virus from a patient to registration of the recombinant vaccine, the development of Hecolin took 14 years (Wu et al. 2012a). Following the product launch in 2012, the vaccine is available in the private market in China, but Inovax is seeking approval of the HEV 239 in Pakistan, Nepal, India, and Thailand (W Huang, pers. comm.).

Further studies of the vaccine are in progress. The safety and immunogenicity of HEV 239 vaccine in healthy individuals infected at baseline with hepatitis B virus (HBsAg-positive)

has been assessed in an exploratory subanalysis in the phase III trial, and found to be similar to that of the general trial population (Wu et al. 2013). It is known that individuals with chronic liver disease (CLD) have a higher risk of severe hepatitis E if infected, similar to the effect of acute hepatitis A or B on patients with CLD, and would therefore be an important target population for this vaccine (Wang et al. 1986; Dalton et al. 2007). Hepatitis A and B vaccines have been found to be safe and immunogenic in individuals with CLD, and are recommended for use in patients without the corresponding infection. A post-licensure study to assess the safety and immunogenicity of HEV 239 in subjects

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with CLD is currently underway in Shandong Province, China with results expected in 2018 (NCT02964910; W Huang, pers. comm.).

In another post-licensure study conducted in China, the safety and immunogenicity of HEV 239 vaccine is being evaluated in healthy individuals over 65 years of age, with results expected in 2018 (NCT02189603; W Huang, pers. comm.). Moreover, a study to evaluate the immunogenicity of an accelerated dosing regimen of the HEV 239 vaccine that might be better suited for outbreak settings was initiated in Zhejiang Province, China. The study will compare the approved vaccination schedule (0, 1, 6 months) with dosing at 0, 7, and 21 days, with results expected in 2019 (NCT03168412; W Huang, pers. comm.). Finally, a study to determine the effectiveness of HEV 239 vaccine in preventing hepatitis E disease among women of childbearing age is ongoing in Bangladesh (NCT02759991). As gt1 HEV is predominant in Bangladesh, this study may provide empiric confirmation that HEV 239 protects an important risk group against disease caused by this vaccine-homologous genotype that is thought to be more pathogenic for humans than gt4.

No studies of HEV 239 vaccine have been conducted in immunosuppressed or immunocompromised patients such as solid organ transplant recipients, HIV-positive subjects, or those receiving chemotherapy.

RECOMMENDATIONS FROM THE WHO

In May 2015, the WHO published a hepatitis E vaccine position paper (World Health Organization 2015). In that paper, the WHO Scientific Advisory Group of Experts (SAGE) acknowledged the significant public health problem posed by hepatitis E, particularly among special populations such as pregnant women and individuals living in displaced persons camps, yet also noted that there are significant data gaps concerning the incidence of HEV infection and disease worldwide. Although Hecolin was considered to be a promising vaccine, the WHO SAGE concluded that there were insufficient data to justify a recommendation for routine use. It was acknowledged that national authori-

ties may decide to use the vaccine based on their local epidemiology, and certain high-risk situations, such as outbreaks, warranted consideration for vaccine use. The WHO SAGE suggested specific areas of additional study for Hecolin, which included immunization of individuals under 16 years of age, the elderly, and high-risk groups such as pregnant women, immunocompromised persons, and those with CLD. In addition, the WHO SAGE suggested that evaluation of vaccine impact in outbreak situations would be valuable, as would exploration of alternative and abbreviated dosing schedules more suitable for outbreaks. Finally, the WHO SAGE noted that vaccine efficacy had only been shown against disease caused by gt4 (World Health Organization 2015).

Since vaccine registration in China in 2011, there has been no use of Hecolin outside of China other than the ongoing effectiveness study in Bangladesh. Although there is great interest among humanitarian aid agencies to deploy this vaccine preemptively in high-risk displaced population camps and hepatitis E outbreaks, several barriers remain. The vaccine has not yet been prequalified by the WHO, a necessary product endorsement that would allow United Nations (UN) procurement agencies to purchase the vaccine for use in health emergency settings. For the WHO to consider a vaccine for prequalification, there must be a set of written standards (Technical Report Series) that detail the required specifications for a product. To date, no such technical documents have been developed for hepatitis E vaccine. Recently, the WHO convened its first meeting to develop these technical documents. Once these technical documents are approved (expected in 2018), a hepatitis E vaccine manufacturer can submit a dossier requesting prequalification. It remains to be seen whether the WHO will prequalify Hecolin for general use or in an emergency setting, in spite of the absence of clinical data concerning protection against hepatitis E caused by gt1–gt3, administration of the vaccine to children less than 16 years of age, or to persons who are immunocompromised, and the sparse data concerning vaccine use in women who are pregnant or breastfeeding. In addition, the current pre-

sentation (bulky single-dose packaging without auto-disable syringes) and the three-dose schedule increase the challenges to widespread use of the vaccine in the most needed settings.

CONCLUDING REMARKS

Here, we have summarized the abundant evidence that hepatitis E vaccine is acceptably safe and effective for prevention of disease caused by HEV, regardless of genotype, in persons 16 years of age and older. We have also discussed the information gaps that exist and steps that might be taken to make vaccination against hepatitis E widely available. Hepatitis E vaccine is approved in China, but there is no recommendation for its use in China's national immunization program as a result of the low incidence of hepatitis E (2.1 cases per 10,000 person-years in the phase III control group [Zhang et al. 2015]) and the complexity of delivering vaccination to persons at increased risk. On the other hand, there are adult populations in low- and middle-income countries in Asia and Africa where the risk of hepatitis E is 100-fold greater, making targeted vaccination programs more cost-effective if the product were available in those countries. Regrettably, the value of hepatitis E vaccination in these populations is not reliably documented. These deficiencies have prevented funding agencies from prioritizing hepatitis E disease and committing the financial support needed to enable greater access to vaccine.

We assert that hepatitis E vaccine can save lives, particularly among women of reproductive age living where hepatitis E is endemic. A value proposition for its use in high-burden settings and a demand forecast must be generated by the international health community while the manufacturer pursues WHO prequalification. We look forward to a future when control of hepatitis E by vaccination is a possibility for those who need it.

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