

Hepatitis E Vaccine Pipeline

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by the

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Executive summary

The hepatitis E virus (HEV) capsid protein is the major target for neutralizing antibodies and most of the experimental vaccines against hepatitis E are based on this antigen. At least eleven HEV vaccines have been evaluated in non-human primates with virus challenge; however, only two experimental vaccines against hepatitis E have progressed to clinical trials in humans. One of these vaccines, termed Hecolin® (HEV 239), is licensed for use in adults in China and is undergoing further clinical evaluation including use in a combined vaccine with human papillomavirus. The second vaccine, termed rHEV (56 kDa protein), sponsored by GlaxoSmithKline, has not undergone further commercial development. The Developing Countries Vaccine Manufacturers Network lists two additional organizations developing HEV vaccines, although these vaccines are currently at the research phase.

Introduction

Due to difficulty and poor yields in culturing hepatitis E virus (HEV), it has not been possible to produce sufficient virus for vaccine production for either live attenuated or inactivated vaccines against this infection. Vaccine development has therefore relied upon preparation of recombinant HEV antigens or DNA for immunization procedures. Potential HEV vaccine antigens have been expressed in a variety of recombinant prokaryotic and eukaryotic systems. Many studies have looked at the immunogenicity of experimental HEV vaccines; in some cases vaccines efficacy has been evaluated using challenge studies with HEV in non-human primates and ultimately human clinical trials.

Experimental HEV vaccines that have been evaluated in non-human primates are described below. Two vaccines have been evaluated in human clinical trials, resulting in licensing of one HEV vaccine. Vaccines being developed by commercial organizations and further innovations by the company that has developed the licensed vaccine are discussed in turn.

Experimental vaccines evaluated using challenge models

HEV is a small RNA containing virus that exists in both enveloped and non-enveloped forms. The HEV genome is a single stranded capped and polyadenylated RNA molecule ~ 7.2 kb in length and encodes three partially-overlapping open reading frames (ORFs). ORF1 encodes several non-structural proteins, whilst ORF2 encodes the 660 amino acid capsid protein. The ORF3 protein seems to be involved in egress of HEV from infected cells.

Most efforts to develop HEV vaccines have focused on ORF2 i.e. the capsid protein, whilst only a single experimental product based on ORF3 has been evaluated as a potential vaccine. The native HEV capsid protein contains conformational neutralizing epitopes (amino acids 459-606) on the surface of the virion corresponding to the P-domain which forms a β -barrel structure. The neutralization sites, preserved between genotypes of HEV, have been elucidated using specific antibodies and studies in non-human primates.

The experimental vaccines include those based on peptides expressed using a variety of prokaryotic and eukaryotic expression systems as well as DNA. These vaccines have been evaluated for immunogenicity and for efficacy using challenge studies with homologous or heterologous HEV strains in non-human primates. Vaccines that have progressed to challenge studies are listed in Table 1.

[trpE-C2 protein](#)

The *trpE*-C2 vaccine consists of amino acids 221-660 of ORF2 of the genotype 1 Burmese HEV strain expressed as a recombinant fusion protein in *Escherichia coli* (Purdy *et al.*, 1992). The expressed protein is recognized by sera from hepatitis E patients as well as HEV-infected non-human primates (Purdy *et al.*, 1992). *TrpE*-C2 was immunogenic in cynomolgus macaques (*Macaca fascicularis*) that received three 80- μ g doses containing aluminium potassium sulphate and prevented hepatitis after challenge by wild-type HEV from either the homologous genotype 1 strain or a heterologous genotype 2 Mexican isolate; in the latter case, however, there was evidence of faecal excretion of the virus, and viral proteins were

detected in the liver though there was no histological evidence of hepatitis. Animals that received only two doses of the vaccine were not protected either against infection or against hepatitis, when challenged with either genotype 1 or 2 HEV strains (Purdy *et al.*, 1993).

There has been no further development of the *trpE*-C2 vaccine.

pE2 protein

The pE2 vaccine consists of amino acids 394-607 of ORF2 of a genotype 1 Chinese HEV strain expressed in *E. coli* (JZ Zhang *et al.*, 2001). The homodimeric form of pE2 is recognized by sera from patients with hepatitis E. The pE2 protein is immunogenic in rabbits (JZ Zhang *et al.*, 2001) and rhesus macaques (*Macaca mulatta*), with antibodies being mainly directed against the dimeric form of the protein (Im *et al.*, 2001). Immunization of rhesus macaques with three 100-μg doses of pE2 together with Freund's adjuvant resulted in protection against infection in most of the animals that were challenged with the homologous genotype 1 HEV strain. Alum-adjuvanted pE2 was poorly immunogenic in mice and deemed unsuitable for further development (Li *et al.*, 2005a).

HEV 239

The HEV 239 vaccine consists of amino acids 368-606 of ORF2 of a genotype 1 Chinese HEV strain (Li *et al.*, 2005a). HEV 239 is expressed in *E. coli* and is similar to the pE2 vaccine except for an extended N-terminal region (Li *et al.*, 2005b). Surface protrusions, formed by dimerization of HEV 239, correspond to a protruding domain of the native virus capsid protein responsible for eliciting neutralizing antibodies. HEV 239 was recognized by sera from hepatitis E patients as well as a panel of monoclonal antibodies including those that neutralize HEV infectivity (Li *et al.*, 2005a). Comparative studies in mice demonstrated that HEV 239 is far more immunogenic than the pE2 protein (Li *et al.*, 2005a) and induces a vigorous T cell response (Wu *et al.*, 2007). HEV 239 is also immunogenic in Rhesus macaques and animals immunized with two 20-μg doses of alum adjuvanted HEV 239 were

protected against hepatitis and infection when challenged with 10,000 copies of HEV of either homologous genotype 1 or heterologous HEV genotype 4 (Li *et al.*, 2005a).

HEV 239 has been evaluated in human clinical trials and this is discussed separately below.

53 kDa protein

The 53 kDa protein consists of amino acids 112-578 of ORF2 of the genotype 1 Pakistani Sar-55 strain expressed in insect cells using a recombinant baculovirus. Analysis of Sar-55 HEV ORF2 expression in insect cells showed that four HEV-ORF2-related polypeptides -- 72 (full length), 63, 56 and 53 kDa in length -- were produced. The 53 kDa protein assembles into virus-like particles, although it forms monomers when purified. It does not contain the main HEV neutralizing epitope, which lies in the region between amino acids 578-607 (Robinson *et al.*, 1998). Immunization of rhesus macaques with two 385-ng doses of alum adjuvanted-53 kDa protein led to induction of anti-HEV antibodies; however, this failed to protect against hepatitis following challenge with 10,000 monkey infectious dose (MID)₅₀ of the homologous Sar-55 strain, although virus replication was reduced (M Zhang *et al.*, 2001). In comparison, the 56 kDa protein which contains the HEV neutralizing epitopes (discussed separately below), was more efficacious against virus challenge in primate models, even with higher infectious doses of the challenge. Consequently, there was no further evaluation of the 53 kDa ORF2 protein.

56 kDa protein

The 56 kDa ORF2 peptide consists of amino acids 112-607 of ORF2 of the genotype 1 Pakistani Sar-55 HEV strain. The 56 kDa peptide is expressed in insect cells using a recombinant baculovirus. The 56 kDa peptide is observed after expression of full length ORF2 72 kDa protein in insect cells and subsequent processing. Unlike the 53 kDa peptide, the 56 kDa peptide does not form particles (Robinson *et al.*, 1998). Immunogenicity of the 56 kDa protein has been demonstrated in cynomolgus and rhesus macaques (Tsarev *et al.*, 1994; Tsarev *et al.*, 1997; Purcell *et al.*, 2003). Initial studies in vaccinated animals demonstrated

protection against hepatitis following challenge with high viral loads of homologous and heterologous HEV strains, although infection was still observed (Tsarev *et al.*, 1994; Tsarev *et al.*, 1997; Zhang *et al.*, 2002). In a larger study, rhesus macaques were immunized with two doses of alum-adjuvanted 1- or 10- μ g of the 56 kDa peptide. Both doses were equally immunogenic and efficacious (more so than a single dose) in the prevention of hepatitis when the vaccinated animals were challenged with 10,000 MID₅₀ of the homologous strain, or heterologous genotype 2 (Mex-14) or genotype 3 (US-2) strains of HEV. In some animals, there was evidence of infection (viraemia) after vaccination and challenge (Purcell *et al.*, 2003).

This vaccine has been evaluated further in human clinical trials which are discussed separately below.

T1-ORF2

The T1-ORF2 vaccine consists of amino acids 126-621 of ORF2 of the genotype 4 Chinese HEV T1 strain (corresponding to amino acids 112-607 of genotype 1 HEV) encoding the 56 kDa protein expressed in Chinese hamster ovary cells (Huang *et al.*, 2009). Previous studies had extensively evaluated the genotype 1 homologue of this protein expressed in insect cells (see 56 kDa protein above) (Tsarev *et al.*, 1994; Tsarev *et al.*, 1997; Zhang *et al.*, 2002; Purcell *et al.*, 2003). Immunization of rhesus macaques with two 40- μ g doses of alum-adjuvanted T1-ORF2 resulted in protection from infection and hepatitis when challenged with 5×10^4 genome copies of virus, regardless of genotype (1 or 4); protection was not observed following challenge with larger viral inocula (Huang *et al.*, 2009). The study demonstrates that a vaccine based on genotype 4 HEV ORF2 protein sequences has similar cross-protective effects to other experimental vaccines based on HEV genotype 1 ORF2 protein. No clinical studies have been performed with this vaccine (Youchun Wang, personal communication).

62 kDa protein

The 62 kDa protein consists of amino acids 112-660 of ORF2 of the Burmese genotype 1 strain expressed in insect cells using a recombinant baculovirus; two HEV-ORF2-related polypeptides (73 and 62 kDa) were produced (McAtee *et al.*, 1996). Cynomolgus macaques were immunized with two 20- μ g doses of alum adjuvanted-62 kDa protein and subsequently challenged with 1,000 MID₅₀ dose of a heterologous Mex-14 genotype 2 strain; two animals were protected from hepatitis, in a third animal, breakthrough infection was observed (Yarborough, 1999). The 62 kDa protein has not been evaluated further.

rHEV VLP

Expression of amino acids 112-608 of ORF2 of the genotype 1 Burmese HEV strain in insect cells using a recombinant baculovirus produces virus-like particles termed rHEV VLP (Li *et al.*, 1997; TC Li *et al.*, 2005). The rHEV VLPs were recognized by sera from hepatitis E patients (Li *et al.*, 1997) and were immunogenic in different animal species following oral administration (without adjuvants) eliciting both systemic and intestinal antibody responses (Li *et al.*, 1997; Li *et al.*, 2001). Administration of five 10-mg doses of HEV rVLPs to cynomolgus macaques protected the animals against hepatitis when challenged with 10,000 MID₅₀ with a genotype 1 Indian strain, although some faecal shedding was observed in one case (Li *et al.*, 2004). There has been no further development of the rHEV VLP vaccine (Tian Cheng Li, personal communication).

pcHEVORF2

The feasibility of DNA vaccination against HEV was evaluated in cynomolgus macaques using plasmid DNA, termed pcHEVORF2, containing the entire Burmese HEV genotype 1 ORF2 sequence (Kamili *et al.*, 2002; Kamili *et al.*, 2004). In an initial study, cynomolgus macaques received four 100- μ g doses of pcHEVORF2 via intramuscular inoculation. The DNA vaccine was immunogenic in all the inoculated animals; however, upon subsequent challenge, using a heterologous genotype 2 Mexican HEV strain, only half of the animals

were protected from infection and hepatitis (Kamili *et al.*, 2002). A further study investigated intradermal administration of pcHEVORF2 in cynomolgus macaques that received four 25-µg doses with or without the use of a gene gun (Kamili *et al.*, 2004). Antibody responses was observed only in the animals immunized using the gene gun and not in those immunized using a similar dose of DNA by intradermal injection. On challenge with a 10-fold higher dose of the heterologous genotype 2 Mexican HEV strain than that used in the previous study, complete protection against hepatitis and infection was observed in animals immunized using a gene gun (Kamili *et al.*, 2004). There has been no further development of the pcHEVORF2 vaccine (Saleem Kamili, personal communication).

Lipo-NE-DP

The Lipo-NE-DP vaccine consists of plasmid DNA containing the neutralizing epitope of HEV (corresponding to amino acids 458-607) of an Indian genotype 1 strain (PM2000), together with the corresponding peptide expressed in *E. coli*, encapsulated in liposomes. Rhesus macaques were immunized subcutaneously with two doses of the liposome-encapsulated DNA (20 µg) and protein (20 µg). Following immunization, the macaques were challenged with the homologous genotype 1 HEV strain containing ~10,000 copies of HEV RNA. Lipo-NE-DP was fully protective and there was no evidence of HEV infection or hepatitis in the vaccinated animals (Arankalle *et al.*, 2009). No further studies on this vaccine have been reported.

ORF3-IL1β

An experimental vaccine has been developed using the entire HEV genotype 4 ORF3 peptide, expressed as a fusion protein with interleukin-1β in *E. coli*, and was shown to be immunogenic in rhesus monkeys that received 40 µg purified protein with aluminium hydroxide adjuvant. Challenge studies, using both homologous genotype 4 and heterologous genotype 1 HEV strains, demonstrated that the vaccine reduced viraemia and faecal shedding although hepatitis was not completely prevented. Antibody to ORF3 in infected people is

relatively short-lived and it appears that there are differences in antigenicity of this protein between HEV genotypes (Ma *et al.*, 2009). This experimental vaccine has not been developed further.

Vaccines evaluated in humans

Two of the most promising vaccines received support to undergo clinical trials in man.

HEV 239

Following successful preclinical studies for HEV 239, an investigational new drug application for phase I/II clinical trials was submitted to China's SFDA in June 2003 and approved in December 2004. The results of these trials were reported to the SFDA in July 2006. In 2007, a phase III trial was approved, whose results were submitted to the SFDA in late 2009. HEV 239 was licensed by the then SFDA in December 2011 and became available on the Chinese market in October 2012 (Zhang *et al.*, 2009; Zhu *et al.*, 2010; Wu *et al.*, 2012; Zhang *et al.*, 2013). Details of the HEV 239 clinical trials are reviewed separately in an accompanying paper titled “Composition, safety, immunogenicity and efficacy of hepatitis E vaccine”.

HEV 239, given the trade name Hecolin®, is manufactured by Xiamen Innovax Biotech Co., Ltd. and is available as a pre-filled syringe containing aluminium hydroxide-adsorbed antigen suspended in buffered saline. The dosing schedule is 0, 1 and 6 months by intramuscular injection. Currently, extended follow up of the phase III clinical trials subjects as well as a small phase IV study involving ~400 subjects of 65 years of age and over are ongoing (Innovax presentation, WHO SAGE hepatitis E vaccine working group meeting, Geneva, 2-4 June 2014). Further clinical trials of Hecolin® and evaluation of a combined HEV/human papilloma virus (HPV) vaccine are planned (Table 2).

56 kDa protein

The 56 kDa vaccine (designated rHEV) was evaluated in phase I clinical trials in humans conducted in the US at Walter Reed Army Institute of Research. The study involved 88 adults

aged 18-50 years; the subjects were divided into groups and received four (1, 5, 20 or 40- μ g) doses of the alum adjuvanted 56 kDa vaccine every two weeks. The vaccine was immunogenic, although the 1- μ g doses resulted in lower seroconversion rates. No serious adverse events were reported, and the vaccine was generally well tolerated (Safary, 2001). The vaccine was also tested in 44 Nepalese volunteers with three doses (0, 1 and 6 months) of either 5- or 20- μ g doses; 43 subjects seroconverted to anti-HEV by the second month and the remaining individual by the seventh month (Emerson and Purcell, 2001).

Subsequently, a phase II randomized controlled clinical trial was undertaken in Nepal, an area endemic for HEV genotype 1. The study involved ~2,000 healthy adults, most (99.6%) of who were men. Subjects received three 20- μ g doses of vaccine at 0, 1 and 6 months and were followed-up for a median of 804 days for clinical hepatitis. The vaccine showed 95.5% efficacy with respect to prevention of hepatitis E, in a per protocol analysis of subjects who received all 3 vaccine doses (vaccine group: 3 infections among 898 subjects; placebo group: 66 infections among 896 subjects), and of 85.5% in an intention-to-treat analysis of those who had received at least one dose (vaccine group: 9 infections among 1,000 subjects; placebo group: 78 infections among 1,000 subjects). The vaccine was well tolerated with local reactions at the injection site being the most common adverse event (Shrestha *et al.*, 2007).

The rHEV vaccine was originally developed by the National Institutes of Health in the USA, and material for the clinical trials was prepared by DynCorp (now Novavax). The same batch of vaccine prepared by DynCorp was used both in the primate challenge studies as well as the human clinical trials. The clinical trials were sponsored by the US army (Walter Reed Armed Forces Research Institute of Medical Sciences) and GlaxoSmithKline. The rHEV vaccine was non-exclusively licensed to GlaxoSmithKline and the company has stated that they would be willing to consider continuation of development of the vaccine through partnerships with other organizations (Innis *et al.*, 2007).

No further clinical trials for rHEV have been undertaken and the vaccine has not been licensed.

Further vaccines in clinical development and future perspective

Table 2 lists HEV vaccines which have entered clinical trials, including the rHEV vaccine and Hecolin® (and variants thereof) as well as vaccines which are under development. The Developing Countries Vaccine Manufacturers Network (DCVMN) has reported that two organizations are at the research phase of development i.e. Cadila Pharmaceuticals Limited in India is working on the 56 kDa peptide expressed in insect cells in association with Novavax, and research is being undertaken on HEV vaccines by the Pasteur Institute of Iran who are undertaking research evaluating recombinant HEV vaccine antigens using bacterial and insect cell-based systems.

Following approval of the first clinical trial for HEV 239, it was a further 8 years before the vaccine was finally licensed in 2011. In the case of rHEV, phase II studies have been completed. Different scenarios concerning the future development of rHEV may be considered. This would include the production of rHEV by different manufacturers which would require studies to ensure comparable quality, immunogenicity and safety of their respective final products compared with the batch of vaccine used in the previous clinical trials. Development of rHEV by the original sponsors of the vaccine may be a more streamlined process; nevertheless, it will be several more years before rHEV or another novel vaccines receives marketing authorization.

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