Hepatitis E Vaccine

Executive Summary

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According to WHO, Hepatitis E virus (HEV) infection is a significant public health problem in many areas of the world. Every year there are 20 million hepatitis E infections, over three million acute cases of hepatitis E, and 70 000 hepatitis E-related deaths. China has produced and licensed the first vaccine to prevent hepatitis E virus infection, although it is not yet available globally. The possibility of a future routine vaccination is still questioned by WHO for public health and economic impact.

Unlike the other agents of viral hepatitis (A,B,C,D), certain HEV strains also have zoonotic hosts. Sporadic HE in non-endemic countries was confirmed as a result of HEV transmission from domestic and even wild animals. Domestic pigs are considered the world wide reservoir of HEV (genotype 3 and 4) infection and the major threat of its transmission to human.

Thus, control of HEV infection especially in low-endemic countries could be achieved with the safe and cost effective way of immunizing pigs and possibly other domestic animals.

Our goal is the development and implementation of the first veterinary vaccine against hepatitis E with our proven technology and novel R&D product.

Our company

NL Biotechnologies is a biotech R&D company that specializes in viral Hepatitis B, C, and E vaccine development. The proprietary recombinant Hepatitis B vaccine technology has been commercialized in 2002 by Instituto Butantan (Brazil) and in 2004 by Probiomed S.A. de C.V. (Mexico). The overall sales exceed 200 million doses. Since 2001, NL Biotechnologies in collaboration with CDC (US, Atlanta) has been developing novel vaccine against viral hepatitis E. At present, we have a prospective candidate completed at lab-scale and ready for trials. NL Biotechnologies is looking for partners in industry for further clinical and commercial development.

Our product

The concept

While ORF2 and ORF3 proteins have been used in many immunodiagnostic assays, ORF2 has been the main target for vaccine development. *In vitro* assay, carried out with serum specimens of mice immunized with bacteria derived ORF2 protein, showed that elicited antibodies have neutralizing properties. Also, the immunization of rhesus and cynomolgus monkeys with ORF2 protein has been shown to protect the animals against HEV infection.

We have developed the yeast strain expressing a truncated ORF2 protein that contains neutralizing epitopes. The protein is expressed in *Hansenula polymorpha* cell system. Our HEV recombinant protein (rHEAg) is glycosylated and self-assembled into virus like particles (VLP) secreted into the culture media. The product reacts with anti-HEV positive human serum specimens. Sera of mice immunized with rHEAg demonstrates specific antibody against ORF2 proteins of different HEV strains (genotypes 1, 2 and 3).

HEV cross-genotype neutralization with serum antibodies was confirmed by the vaccination with genotype 1 capsid protein that protected rhesus monkeys from challenge by HEV viruses of other mammalian genotypes (Purcell et al., 2003). Thus, our genotype 1 rHE-Ag can elicit protective immunity in pigs and other animals against genotypes 3 and 4. At the same time, it still remains a prospective candidate for future human safe and cost-effective vaccine for endemic countries.

Our results show that the truncated HEV capsid protein expressed in *Hansenula polymorpha* yeast system has the immunogenic properties of a prospective candidate for the vaccine development.

Technology

The technology is based on the expression of HEV protein, corresponding to position 452-617 aa Open Reading Frame 2 (ORF2- genotype 1) in Hansenula polymorpha yeast cells using methanol oxidase (MOX) promoter.

Following yeast transformation and confirmation of successful plasmids integration, the culture of yeast cells is forced to produce the target protein combining both: cells growth and induction of promoted expression. These two processes run simultaneously following our original technology of fermentation.

Since the target protein is not accumulated inside the cells and is secreted into the fermentation media, no cell extraction is required. The broth is centrifuged and the supernatant is used for the rHEAg-VLP purification. More than 95% of purity is achieved by only ultrafiltration and ion-exchange chromatography.

Conclusion

HEV infection of swine, and possibly other animals, is common throughout the world, and animal strains provide a reservoir for sporadic zoonotic infection. HEV was detected in commercial pork products throughout the whole Europe. In addition, seropositivity for HEV was associated with consumption of raw or undercooked pig livers, with being a swine worker, and with wild boar consumption.

We have demonstrated that our transformed Hansenula polymorpha cells are able to express and efficiently secrete recombinant protein of HEV (rHEAg) into the cell culture fluid in the form of VLP. It is further interesting that the product is expressed in glycosylated form, which may assist higher immunogenicity.

The full version of dossier is available reflecting the performed work for the development of HEV experimental vaccine on pilot scale. The laboratory samples of rHEAg are available and can be provided upon request. The samples may be used for analysis and in experiments with animals.

The technology is ready for transfer to a designated facility for scaling up and adaptation to GMP requirements. If necessary, the technology can be demonstrated in the designated facility by a team of two NL Biotechnologies specialists in the course of two weeks.

Intellectual property rights for this vaccine technology are protected by patent pending in Russia. International patent application is in progress.