Executive Summary

New HCV Vaccine Research and Development

www.nlbiotechnologies.com

About 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 vaccines against viral hepatitis. At present, we have several prospective candidates completed at lab-scale and ready for trials: hepatitis B prophylactic third generation vaccine (HBs-PreS1-PreS2), therapeutic hepatitis B fourth generation vaccine (HBs-HBc-PreS1-PreS2), two hepatitis C vaccine candidates and hepatitis E vaccine candidate. Currently, NL Biotechnologies is looking for partners in the industry for further clinical and commercial development.

About our products

Overview

Hepatitis C virus (HCV) is an enveloped, positive-stranded RNA virus from the Flaviviridae family. Its genome encodes a single polyprotein of 3,000 amino acids, which is cleaved into different viral proteins by viral and cellular proteases. HCV is highly variable and known for developing populations of quasispecies within the host. Three structural proteins have been identified: the core protein and two envelope glycoproteins, called E1 and E2. E2 harbors HVR1 in its N-terminus that encodes a 27-31 amino acid sequence, and which itself is one of the main contributors of the genetic variability in the quasispecies populations.

HCV is regarded as a major human pathogen. It is currently the main cause of chronic liver disease, and it frequently leads to cirrhosis and liver cancer. It has been estimated that 170 million (3%) persons worldwide are chronically infected with HCV and that there are 3-4 million new cases each year. Although most acute HCV infections are asymptomatic, more than 75% of cases will result in chronic hepatitis. A major burden of HCV infection is the chronic liver disease that develops asymptomatically over several decades. In 25% of cases, this chronic disease results in cirrhosis and/or hepatocellular carcinoma, which in turn lead to the death of more than 350,000 people each year.

The transmission of HCV infection predominantly occurs via the blood, and most incidences are linked to the sharing of injection equipment. However, the identification of increasing numbers of recent outbreaks resulting from healthcare-associated exposures is a significant concern, and the population burden of such exposures remains unclear.

Antiviral therapy provides a sustained response in about 50% of treated persons, with some HCV genotypes responding better than others. However, the treatment is not always well-tolerated, and

relatively few infected persons initiate and complete treatment. Despite ongoing research, there is no vaccine available to prevent HCV infection.

Intellectual Concept

Our HCV vaccine project is divided in two main branches. The first one is the development of a prophylactic vaccine that will target the HCV E2 HVR1 epitopes, exploring the cross-reactivity of antibodies raised against that region. We constructed nine yeast-derived hybrid proteins, each containing a different HCV HVR1 variant inserted at the N-terminus of the a-determinant of recombinant HBsAg.

Several studies demonstrated the integration of HCV HVR1 into recombinant HBs-Ag. However, this integration completely inactivated the immunogenicity of HBs, thus allowing the structure to function exclusively as a carrier for the foreign epitopes.

Our HBs-HVR1 constructions are different because the a-determinant antigenicity is preserved by the insertion of the HVR1 near the N-terminus, which allows this structure to serve as a potential bivalent vaccine against HBV and HCV.

Additionally, because HVR1 sequence variability has been a limiting factor in the development of HC vaccine, immunization with a mixture of these hybrid proteins should offer a broader protection. We called this project "**Project A**".

The second project involves the construction of nine HCV-like particles (HCV-VLP), each of which contains the three HCV structural proteins (core, E1 and E2) also with a different HVR1 in each construction. Such VLPs are attractive as recombinant protein viral vaccines because they closely resemble the native virion form in terms of their morphological, biophysical and immunogenic properties.

In addition to commonly considered E2, the use of the HCV E1 protein in prospective HC vaccines has recently been gaining attention. Several studies have suggested that both the humoral and cellular immune responses elicited by the E1 protein are impaired in patients with chronic HCV. The characteristics of the E1 protein, such as its degree of inter-genotype cross-reactivity and its relatively high degree of conservation between some subtypes, make it a good target for vaccine development.

Finally, besides the induction of a strong humoral response, an efficient vaccine against HCV infection will also require the elicitation of a broad cellular immune response. The spontaneous eradication of HCV is associated with a strong $CD4^+$ and $CD8^+$ T cell responses against the HCV core protein. The HCV core protein has been recognized as the most conserved region of the translated HCV genome in all genotypes and thus as the prospective candidate for vaccine formulation.

We expect our HCV-VLP containing all three proteins (E1, E2/HVR1 and core) to function as both prophylactic and therapeutic vaccine. This project is called "**Project B.**"

Technology

Project A – HVR1-HBsAg

The technology used in this project is based on recombinant DNA technique and yeast genetic methods. The plasmids are constructed to encode hepatitis B surface protein (HBs) with selected HCV E2 HVR1 and then are inserted into *H. polymorpha* for protein synthesis.

Following the yeast transformation and confirmation of successful plasmid integration, the yeast cells are forced to produce the target proteins using our original fermentation technique, where we run cell growth and the induction of expression simultaneously.

When the expression process is concluded, we initiate the purification process. Because more than 90% of the total protein weight corresponds to HBs, and only 10% corresponds to HVR1, the downstream purification technology is very similar to the HBsAg technology that has already been successfully established by Dr. Nikolai Granovski in Brazil and Mexico.

Given the high capacity of the HBsAg as a vector/carrier and the small relative size of the HVR1 insertions, we believe that the properties of the HBsAg, with the inserted constructs, are not altered. Similarly, experiments have shown that HBs-HVR1 hybrids successfully pass through all stages of the HBsAg purification technology. However, this technology may not be suitable for direct use for new products. More research regarding and refining of the fine-antigen purification step are required. Furthermore, for new products, we must develop and standardize quality control steps based on their specific properties.

Project B – HCV-VLP (HVR1)

The technology for Project B is also based on the recombinant DNA techniques that were described in Project A. For Project B, the plasmids were constructed to encode HCV structural proteins (core, E1 and E2/HVR1), which were also suited for protein expression in *H. polymorpha*.

Again, the transformed yeast was forced to produce the target proteins by combining the cell growth and induction of target protein expression processes. These two processes ran simultaneously, using our original fermentation technology. When the target protein expression process was concluded, the downstream and purification processes were initiated.

Because the target proteins remain inside the cells, the biomass obtained must be disrupted and the internal cell contents released by pressure extraction. After the cell extract was separated from the cell debris, the purification process was initiated.

Because the target product is a VLP, several physical methods were used to isolate large-molecular-weight proteins. The accepted purity rate was 90%, and because of the unique structure of the product, which is a single polyprotein, special efforts were required for its proper characterization.

Conclusion

The original approach was used for the creation of two prospective candidates for new HC vaccine . We identified a set of HVR1 sequences (in cooperation with the Centers for Disease Control and Prevention (CDC), Atlanta, USA), that when used in combination, showed up to 90% immune cross-reactivity to a HVR1 peptides set, which is a representation of all major HCV HVR1 variants. These sequences were subsequently used in two VLP models: (A) the chimeric version, which uses the surface antigen of hepatitis B virus as a carrier (HVR1-HBsAg) and (B) by expressing the three HCV structural proteins and exchanging the HVR1 in each construct. Both VLP types were highly expressed in the yeast *H. polymorpha*, and the resulting protein products were isolated and purified from the yeast. In addition to eliciting a humoral response against HVR1 in mice, the products from Project A also conserved the anti-HBsAg immunogenic properties, which suggests that these products are candidates for a bivalent vaccine against HCV and HBV infections.

Products from Project B included variants of HCV-LP, each of which bore a distinguishing HVR1 sequence. These HCV-LPs are prospective prophylactic and therapeutic vaccine candidates because of their potential to induce virus-neutralizing humoral immunity and strong cellular immunity.