Development of a cost effective Romania-Norway joint plant-based technology platform for production of vaccines against Human Hepatitis viruses B (HBV) and C (HCV) (GreenVac)
Phase 2-2015 Expression, characterization an purification of the HBV and HCV antigens

Summary

At this stage of the project all 9 antigens proposed within the project and cloned during the phase I (2014), comprising 3 HBV and 6 HCV antigens were produced. The viral proteins were successfully obtained both, in plants (tobacco and lettuce- P1), using two complementary expression technologies (transient and nuclear transformation) and mammalian cells (HEK- PP) as reference and control.

The antigens were characterized biochemically and functionally, considering several features that are important for the immunogenic properties of a protein (PP, P3, P4)

The 3 HBV antigens, all derived from the "small"-S envelope protein are able to assemble within macromolecular structures resembling the subviral particles (SVP), which appear larger in size when expressed in mammalian, compared to the plant system, as resulting from the Asymmetric Flow Field Flow Fractionation (P2). This feature could be due to the different chemical composition of the plant and mammalian intracellular membranes, which could impact on the stability of larger-sized SVP. This interesting observation will be investigated by alternative methods (electronic microscopy) during 2016. As far as the antigen glycan structure is concerned, the glycosidases treatment has shown that the plant and mammalian cell- produced proteins have similar N-linked, high mannose structures (PP).

An important progress of the project was represented by the optimization of a rapid and simple HBV antigen purification protocol, based on the property of these proteins to form SVP (PP). The protocol was scaled-up to produce large amounts of these proteins, required for the immunization studies.

The HCV proteins were readily expressed in mammalian cells (PP), however, their detection in tobacco and lettuce was lower (P1). It is likely that the E1E2 viral polypeptide proposed as "core" antigen in this project is less efficiently processed in vegetal cells. Nevertheless, our preliminary data shows that the lettuce-expressed HCV antigens accumulate in sufficient amounts to conduct the immunology studies, as in this case protein purification is not necessary (animals are fed with the edible plant). In the case of tobacco expression, two alternative expression solutions were proposed within the consortium that will be applied in 2016.

The biochemical and functional analysis of the HCV antigens have shown that removal of the N-Glycosylation sites 1,4, 6 and 11 from the E2 protein- as proposed in the project, does not affect the correct folding or the dimer assembly of the antigens and their interaction with CD81, a major HCV cell receptor (PP, P3, P4).

Similar to HBV, a protocol for the purification of the HCV antigens was optimized (PP), based on the affinity of the *Galanthus nivalis* lectin to interact specifically with high mannose N-linked sugars attached to the proteins.

Based on the results from the biochemical and functional characterization, as well as the purification yields, the HBV/HCV antigens with best properties were selected for the immunization studies.

An important progress of the project is represented by the optimization of the antigen immunization schemes, using one antigen of each HBV/HCV series as a model (P2). The analysis of the immunological markers has demonstrated their immunogenic properties. This study has also established the amount of antigen required for the immunological investigations and that of the biological material (plant or mammalian cells) to start with (P2, PP)

The results obtained so far were disseminated at 3 international conferences, receiving a great interest from the academic community. A publication plan for 2016 was established within the consortium. The studies involved in this project are the subject of a PhD thesis; the PhD student has successfully defended the PhD project plan for the 1st year of the thesis.

In all, we appreciate that the major objectives of this phase of the project were accomplished, each partners fulfilling the activities assigned and participating at the common ones.