**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)**

**Phase 3 (2016): Expression, characterization and purification of the HBV/HCV antigens**

Summary

Based on molecular and functional characterizations performed during the previous stage of the project, the selection of designed HBV and HCV antigens for the immunological studies was completed in the current stage (PP, P1, P3). The optimized protocol for the purification of viral proteins was expanded at large scale and successfully used in order to obtain sufficient quantities of antigens to carry out studies on animals (BALB/c mice) (PP). The immunization experiments were performed using protocols optimized in the previous stage (in case of injected antigens), and new protocols were also developed (in case of oral antigens and mixed deliveries) (P2). The antigens efficiency was investigated by detailed analysis of the immune response at humoral and cellular levels, in vaccinated animals (PP, P2). The ability of the immune sera to neutralize the HBV/HCV infection *in vitro*, using cellular systems specific to each virus was also investigated (PP, P3, P4). The experiments carried out are summarized in the table below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antigen** | **Description** | **Selection reasons** | **System of expression** | **Vaccination route** |
| HBV-S | The HBV S wild-type protein*,* ayw genotype | -stable protein  -high production  -efficient purification  -reference system (constituent of the standard vaccine) | Mammalian cells HEK293T | Injection |
| Plants, *Nicotiana benthamiana* | Injection |
| Plants, *Lactuca sativa* | Oral |
| HBV-Ins  (HBV-S/preS121-47) | HBV S protein containing the pre-S1 sequence inserted in the AGL domain | -stable protein  -high production  -efficient purification  -new antigen | Mammalian cells HEK293T | Injection |
| Plants, *Nicotiana benthamiana* | Injection |
| Plants, *Lactuca sativa* | Oral |
| HBV-Del  (HBV-S∆127150/preS121-47) | HBV S protein where the AGL domain was replace by the preS1 sequence | -stable protein  -medium production  -secretion deficiency  -new antigen  -control used to recognize the preS1epitope | Mammalian cells HEK293T | No |
| Plants, *Nicotiana benthamiana* |
| Plants, *Lactuca sativa* |
| HCV-E1E2 | Wild-type polypeptide HCV E1E2*,* 1a genotype | -stable protein  -high production  -efficient purification  -main candidate to develop a new vaccine | Mammalian cells HEK293T | Injection |
| Plants, *Lactuca sativa* | Oral |
| HCV-E1E2∆N6 | Polypeptide HCV E1E2, containing the deletion of the N-linked glycosylation at position 6 (genotype 1a | -stable protein  -high production  -efficient purification  -new candidate for a future vaccine | Mammalian cells HEK293T | Injection |
| Plants, *Lactuca sativa* | Oral |
| HCV-E1E2∆N11 | Polypeptide HCV E1E2, containing the deletion of the N-linked glycosylation signal at position 11, (genotype 1a) | -stable protein  -high production  -efficient purification  -new candidate for obtaining of a vaccine  -representative for the N-glycosylation mutants | Mammalian cells HEK293T | Injection |

The immune sera collected from vaccinated mice were subjected to specific analyses to evaluate the humoral and cellular immune response. The data obtained for each antigen, purified from either animal cell cultures or plants will be comparatively analyzed to identify the antigen with best immune properties of each HBV/HCV series.

The results obtained at this stage of the project were disseminated by the members of the consortium at 7 international conferences, receiving an excellent feedback. The PhD student working on this project has successfully defended her 2nd -year Research Project-progress report, receiving the maximum grade “Very well” from the examination committee. The first two manuscript resulted from the project have already been submitted to high-impact factor journals, Plant Biotechnology Journal, IF =6 and Scientific Reports, IF= 5,1, another manuscript being currently under preparation.

We estimate that the project objectives and indicators have been entirely fulfilled, each partner has performed the tasks assigned participating in common activities as well.

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**Phase 4 (2017): “Evaluation of the immunogenic properties of the HBV and HCV antigens; the socio-economic impact of the proposed antigens”**

Summary

At this last stage of the project, the work was focused on analysis of the immunology data resulted following vaccination of the BALB/c mice with animal cell culture- or plant-derived HBV/HCV antigens, obtained in previous year. The data have shown that HBV-Ins (HBV-S/preS121-47), the novel HBV antigen designed and produced within this project, has significantly better immunogenic properties than HBV-S, the protein of the standard, commercial vaccine produced in yeasts. Importantly, the antibodies obtained by vaccination recognized both S as well as preS1 epitopes (PP, P2); moreover, this immune serum neutralized the HBV infection in cell culture more efficiently than that obtained by vaccination with HBV-S (PP). These remarkable properties recommend the HBV-Ins antigen as an important candidate for the production of an alternative to the current vaccine and the plant-based production as a valid and economical platform for production of this type of chimeric antigen.

Regarding the HCV antigens, it was proven for the first time, the successful production of the E1E2 dimer in plants and its capacity to trigger formation of neutralizing antibodies, in case of mixed, injected and oral delivery. Deletions of the N-glycosylation sites seem to improve the antigenic properties of the dimer (at least in case of ∆N6), however, detailed studies of the sera obtained and the description of antibodies- E1E2 dimer (various genotypes) interactions are required to validate these data (PP, P2). Notably, cloning of the HCV E1E2 dimer from HCV- infected patients at various stages of the diseases was finalized successfully and viable, infectious HCV pseudoparticles (pp) were assembled to enable cross-genotype neutralization studies (P3).

The socio-economic analysis was performed (P1) and end-users views on the potential vaccines produced in this project, as well as the standard vaccines available or the recombinant vaccines produced in nonedible and edible plants were recorded following interviews with patients and medical staff (P1, P3).

The results dissemination has continued at this phase of the project, a new manuscript being submitted to Antiviral Research, IF= 4,9 (currently under revision). Three other manuscripts are also in preparation and will be submitted as soon as analysis of all immunology data available will be finalized.

The project closing conference was organized with participation of the project members, other scientists interested in this research area, officials from the contracting authority and mass- media (PP).

We believe the activities of this pahse of the project have been carried out according to the project plan, general and specific objectives, as well as project indicators being fully achieved.