Project summary- I Phase July- December 2014

During the first stage of the project, management and consortium organization activities were carried out, as well as experimental procedures. These aimed to obtain the novel HBV and HCV antigens assumed in the project, using specific molecular biology techniques and clone them in mammalian and plant expression vectors. The project activity plan also comprised the expression and purification of the newly obtained antigens; however these complex experiments will be concluded in the next steps of the project, as designed.

Due to the excellent collaboration between **PP-CO, P3 and P4,** all eight proposed antigens (two for HBV and six for HCV) were obtained using different mutagenesis and cloning strategies, which are detailed in *the extenso* research report.

The DNA sequences encoding for these novel antigens were sent to Norway at Bioforsk **(P1),** where our partners cloned them in specific vectors designed for plant expression of exogenous proteins. At this stage of the project, the Bioforsk team has opted to introduce the antigens sequences into agrobacteria (*Agrobacterium tumefaciens),* used to infiltrate tobacco (*Nicotiana benthamiana)* leaves. This system ensures the transient expression of the proteins of interest, required for a first evaluation of the viral antigens biosynthesis yield, as well as of their properties. Two of the proposed antigens have already been synthesized in tobacco leaves, the procedures for the remaining antigens being currently ongoing. Following the detailed biochemical and functional characterization of the antigens obtained, we will continue with their stable expression in tobacco and edible plants (lettuce).

The **PP-CO** has continued in parallel the characterization of the novel antigens produced in mammalian cells, which will be used as reference and control systems. The viral proteins were found to be viable, with an expression level similar to that obtained for the wild-type proteins. One of the HBV antigens carrying an important deletion at the level of the S envelope protein has shown a weaker expression in transfected mammalian cells. In this particular case, other characterization methodologies will be employed to establish whether its synthesis or assembly are deficient, in which case co-expression and co-assembly experiments using the wild-type S protein (trans-complementarity) will be performed.

Thus, we estimate that all objectives of the current project designated for the year 2014 have been fulfilled, our consortium is functional and each partner has carried out the assigned tasks and has participated at common activities in accordance with the project plan. It is important to note that **P2** was not involved in the 2014 activities and therefore, did not receive funding this year.