**REA_13 - Development of a new multiepitope protein of hepatitis C virus for diagnostic purposes**

Mayara Torquato Lima da Silva[1]*; Livia Melo Villar[1].
[1]Fiocruz/IOC.

**Introduction:** Infection with the hepatitis C virus (HCV) is a serious public health problem worldwide. One of WHO's 2030 agenda goals is the elimination of hepatitis C and to achieve this goal, one of the strategies is to expand access to diagnosis of positive cases that are often unidentified mainly in vulnerable populations. Current ELISA assays and immunochromatography tests (fourth generation) use HCV recombinant viral antigens from various structural and non-structural protein peptides. One of the rapid tests currently adopted by SUS uses antigenic fractions from Core, NS3, NS4 and NS5 to identify anti-HCV. An analogous alternative is the production of recombinant multiepitope proteins from HCV immunogenic peptides as an attractive approach to express only a single functional and more sensitive protein.

**Objective:** Design a new HCV multiepitope protein capable of representing the multiple genotypic variables of the virus prevalent in Brazil and that can be subsequently used for the preparation of rapid tests for the diagnosis of hepatitis C.

**Methodology:** The HCV multiepitope protein was designed using immunogenic peptides from HCV proteins (core, E2, NS3, NS4, NS5A and NS5B) from genotypes 1a, 1b and 3a. The regions were selected based on pre-existing information in the literature and designed using the tools T Cell Epitopes - MHC Binding Prediction (IEDB Analysis Resource©) and VaxiJen v2.0 for epitope prediction. Alignment between immunogenic peptides and HCV nucleotide sequences from previous studies by the Laboratório de Hepatites Virais was also carried out in the MEGA v. 7.0 to confirm whether selected regions would meet local genotypic prevalence. The gene was synthesized in the expression vector pET21a with codon optimization for expression in *E. coli*. The stability of the recombinant protein was evaluated *In silico* by molecular modeling on the online server I-TASSER and the tool "GalaxyWEB Refine" for the refinement of the three-dimensional structure.

**Results:** The gene has regions of the three most prevalent genotypes in Brazil and the peptides selected to compose the HCV multiepitope protein are conserved among the sequences of the local reference panel. The protein also reached an average score of 0.56 by the VaxiJen v2.0 tool, which indicates the antigenicity of the molecule. Structural prediction by molecular modeling demonstrated that the protein in solution and in stable thermodynamic conditions assumes preferential secondary structures of α-helix in accordance with the structural models used as a template by the I-TASSER server.

**Conclusion:** From the tools used, it was possible to design a new gene encoding a HVC multiepitope protein. The observed immunogenicity score indicates that the protein must be able to develop an immune response in *in vivo* assays and recognize anti-HCV antibodies, which makes it suitable for use in diagnostic tests for hepatitis C.

**Keywords:** Multiepitope protein; HCV; molecular modeling