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# AN *IN SILCO* STUDY ON IMMUNOGENICITY DOCKING OF DISEASE CAUSED ANTIGENS IN HPV TYPE16 [STRAINS E6, E7]

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**ABSTRACT : Vaccines work by mimicking disease agents and stimulating the immune system which in turn Builds a defense mechanism against the disease-causing agents. Some of the vaccines contain a part of the disease-causing agents which are either weakened or dead. Apart from using vaccines only for viral infections, utilizing the same against Cancers both as therapeutic and preventative has captured huge interest. The use of Cancer vaccines in cancer therapies is called immunotherapy, which is done either by specific cancer vaccine or universal cancer vaccine which contain tumor antigens that stimulate the immune system which in turn initiate various mechanisms that terminate tumor cells and prevents recurrence of these tumors. Here, we conduct some in silico approaches to select best strain target proteins we perform strain selection, epitope prediction, antigenicity, immunogenicity prediction and docking of target proteins to find out the best targets for further studies.**

***Key words :*** HPV type 16 [strains E6, E7], ligands, pymol, chemsketch, gold, docking.

## INTRODUCTION

The human papillomavirus (HPV) is common diseases of sexually transmitted infectious in male and female in the world, in particularly in developing countries, where the prevalence of asymptomatic infection different from 2 into 44%, depending on the population (Sanjosé *et al*, 2007). Some studies show that HPV infects the sexually active persons (Baseman and Koutsky, 2005; Trottier and Franco, 2006). HPV infection is more active in young adults, then it decreases with age progress (Castle *et al*, 2005; Fernandes *et al*, 2009; Chan *et al*, 2010). HPV can infect basal epithelial cells of the skin or inner-lining tissues and are categorized. Cutaneous types are epiderm tropic and infect the keratinized surface of the skin, targeting the skin of the hands and feet. Mucosal types infect the lining of the mouth, throat, respiratory, or anogenital tract epithelium (Burd, 2003). About 40 different HPV types can infect the epithelial lining of the anogenital tract and other mucosal areas of the human body. HPVs can also be classified as high-risk (HR-HPV) and low-risk (LR-HPV) oncogenic types based on their association with cervical cancer. LR-HPV types, such as HPV 6 and 11, while infection with HR-HPV types, highlighting HPV 16 and 18, is associated with the occurrence of pre-malignant and malignant cervical lesions (Muñoz *et al*, 2003; Bosch *et al*, 2002; Bosch *et al*, 2008). HR-HPV types are also associated with many penile, vulvar, anal and head and neck carcinomas and contribute to over 40% of oral cancers (Stanley, 2010).

The critical molecules for initiation and progression of this cancer are the oncoproteins E5, E6, and E7, that act largely by overcoming negative growth regulation by host cell proteins and by inducing genomic instability, a hallmark of HPV associated cancers (Munger *et al*, 2004; Moody and Laimins, 2010). Once HPV transmission to the genital tract occurs through sexual contact, the risk factors for the infection and cervical lesions, including cervical cancer are the same classic risk factors for other sexually transmitted diseases. In addition, other indicators of sexual behavior and reproductive activities, heredity, immune and nutritional status, and smoking can contribute in some way to the development of cervical cancer (Muñoz, 2006; Fernandes *et al*, 2010).

### Functions of viral proteins

### E6 protein

The HPV E6 protein is composed of around 150 amino acids and consist of two fingers like zinc connected by linker between domains of 36 amino acids, surrounded by terminal domains short (N) amino and (C) carboxy range of different lengths (Howie *et al*, 2009). The good

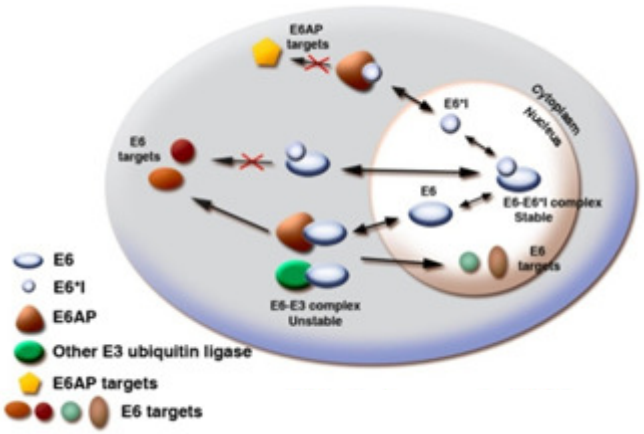


**Fig. 1 :** shows role of E6.

protein called E6-targeted protein 1 (E6TP1) in an E6-AP based approach (Wooldridge *et al*, 2007), tuberin, that can too be bound and degraded from E6 (Zheng *et al*, 2008). Moreover, HR-HPV E6 was found that react with two proteins, which are portion of the innate immune response to viral infection: interferon regulatory factor-3 (IFR-3) and toll-like receptor 9 (TLR9) (Hasan *et al*, 2007). External expression of HPV16 E6/E7 was found that prevent TLR9 clone, driving to a functional waste of TLR9 signaling roads inside the cell (Hasan *et al*, 2007).

HR-HPV E6 is too capable that react with members of the PDZ family of proteins, enhancing its proteosome-mediated degradation, an efficacy which seems to be

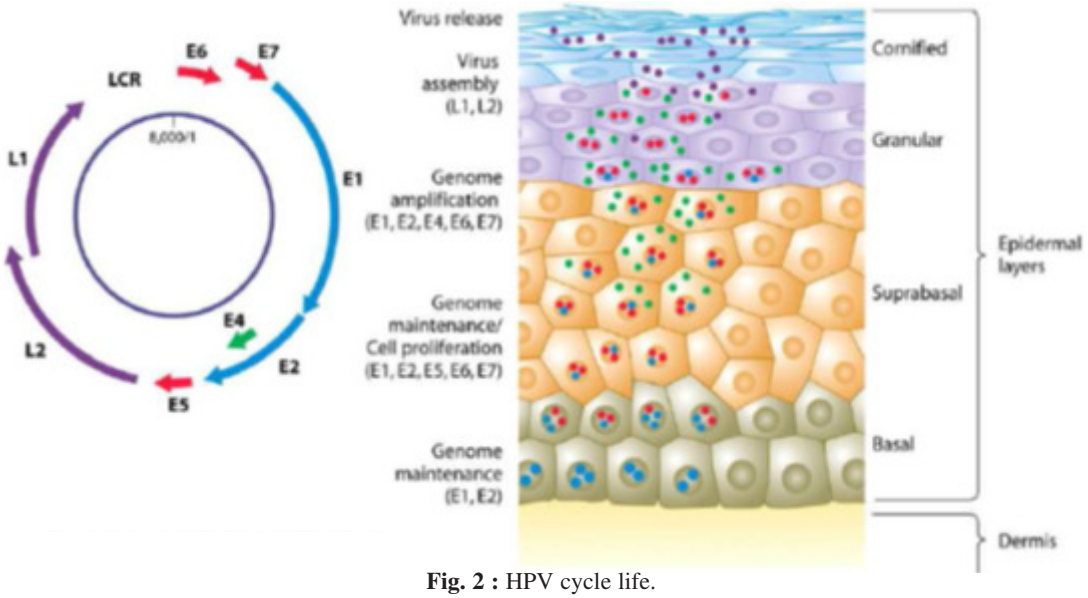


**Fig. 2 :** HPV cycle life.

advantage of E6 proteins of HR-HPVs is capacity to combine and resolve the tumor suppressor of p53, during the mobilization of the E6-associated protein (E6-AP), a cellular E3 ligase, which does not combine to p53 in the lack of E6. Both E6 of HR-HPV and LR-HPV combine p53, but the reaction is different levels (Lechner *et al*, 1994).

Three different actions have been expected to explain p53 inactivation: The first is inactive the binding of p53 – in the genome - to its target, second, E6 perhaps capacity to inaction p53 signaling by keeping of p53 in cytoplasm; and third, the action supplied by E6 for inhibit p53 action is the transactivation revocation of p53 responsive genes by reaction with CBP/p300 or hADA3 histone acetyltransferases (Kumar *et al*, 2002).

E6 is capable to modify clones from other cellular signaling ways as long as potentiating its capability to act as a vary modifier for host cell signaling. It was found that E6 react with three various proteins, like a novel needed for cervical carcinoma induction (Shai *et al*, 2007). LR-HPV E6 does not include the PDZ-linking motif and thus cannot aim these proteins. PDZ proteins disintegrate results in cellular transmutation because lost of cell-cell contact and lack of cell polarity (Storrs and Silverstein, 2007).

Another task of the HR-HPV E6 protein which is significant for immortalization is their capacity to activate the expression of the catalytic subunit of telomerase (hTERT). Therefore, the E6 protein is capable to enhance the telomere repair, during the telomerase work (Hamid *et al*, 2009).

## E7 protein

The E7 protein has approximately hundred amino acids in length and includes three preserved areas: CR1, CR2 and CR3 (Münger and Howley, 2002). It will stimulate cellular propagation by linking of several cellular factors. The connecting of high-risk E7 to pRB damages the reaction between pRB and E2F, a family of

genes of cloning , which led to the constitutive expression of E2F-responsive genes, like cyclin A and cyclin E and enhances early S phase entry, DNA synthesis, and the progression of cell cycle (Zerfass *et al*, 1995). So, the expressing in cells the HPV E7 protein, this checkpoint control at G1/S transition is lost and the cells will continue their cell cycle, causing an uncontrolled cellular proliferation (Jo & Kim, 2005; Huh *et al*, 2007).

E7 has another active which participates to cellular immortalization is its reaction with the CDK inhibitors (CKI) p21 and p27, efficiently neutralizing their inhibitory effects on CDK2 activities, an important factor for G1 to S phase entry and progression (Moody and Laimins, 2010).

High-risk E7 has further was found that increase the levels of the CDC25A phosphatase, which can induce tyrosine dephosphorylation of CDK2, enhancing its activation (Moody and Laimins, 2010).

Moreover, E6 and E7 interfere with the effects of different growth prevent cytokines that are induced following infection. High-risk HPV proteins suppress the transcription of several IFN-inducible genes (Kanodia *et al*, 2007; Tindle, 2002).

**Life cycle of HPV**

The life cycle of HPV begins with stem cells infection in the epithelium basal layer. The virus entry into the cells, it requires the expression of E1 and E2 genes to preserve a low number of genome copies. These proteins bind to the viral origin of replication to enlist cellular DNA polymerases and another protein essential for replication of DNA (Hamid *et al*, 2009). The expression of genes E1, E2, E5, E6 and E7 participates into the maintenance of the viral genome and encourages cell generation, increasing the number of HPV-infected cells in the epithelium, resulting in a higher number of cells that will ultimately produce contagious virions, these process take place in the suprabasal layer (Hamid and Gston, 2009; Lazarczyk *et al*, 2009). The activation of differentiation-dependent promoter and maintenance of gene expression E1, E2, E6 and E7 take places in the more differentiated cells of this same layer of the epithelium (Nakahara *et al*, 2005; Lazarczyk *et al*, 2009). For a better understanding, the HPV life cycle was divided into two parts: a maintenance phase and differentiation-dependent phase (Bodily and Laimins, 2011).

**1. Maintenance Phase**

HPV virion infects cells of the basal epithelial layer, which become uncovered throughout micro lesions. The viral capsids bind firstly into the basal cell layer (Kines *et al*, 2009). HPV genomes reproduce in the nucleus of the basal cell layer, where the virus establishes itself as a low-copy-number episome by using the host DNA reproduction machinery (Moody and Laimins, 2010). In this pathway, viral proteins are expressed at very low levels in undifferentiated cells (Bodily and Laimins, 2011).

The viral episome maintenance in basal cells is the requisite function of the early or maintenance phase of the viral cycle. The expression of E6, E7, E1, and E2 are necessary for persisted episomal maintenance. E1 and E2 collaborate to initiate viral DNA reproduction, whereas E6 and E7 modulate cell-cycle regulators to maintain long-term reproduction efficiency (Conger *et al*, 1999). The E2 protein is perhaps a major regulator of this process because it is able to make both positive and negative control of the early viral contractor that regulates expression of E6, E7, and E1 as well as E2 itself (Steger *et al*, 1997).

Establishment phase is following stage, where viral DNA is reproduced coordinately with host cell chromosomes and virus genomes are spread to the daughter cells. However, in the differentiated keratinocytes of the suprabasal layers of the epithelium, the virus switches to a rolling-circle mode of DNA reproduction, expanding its DNA to a high copy number, synthesizing capsid proteins and collecting the viral particle (Flores *et al*, 1999).

HPV reproduction begins where the host cell factors react with the LCR region of the HPV genome and start the early viral genes transcription (Syrjânen and Syrjânen, 1999).

**2. Differentiation-dependent phase**

During the previous phase of undifferentiated cells, viral proteins are expressed in weak levels. While, when HPV-infected cells depart the basal cells layer, they subject differentiation and high levels of viral proteins create are induced. This restriction of viral protein create in highly differentiated cells protracts the viral antigens expression to locations minimal susceptible to the host immune response (Frazer, 2009).

The viral protein E7 is responsible for preserving the reproduction efficiency in differentiated cells and this is done by inactivation of pRB family members (Münger *et al*, 2004). The late viral promoter activation in response to host-cell differentiation happens in the surround of the spinous epithelial layer and is responsible for high levels of viral protein expression. Approximately an outcome, the virus copy-number amplifies about 50-200 copies to many thousands of copies per cell (Bedell *et al*, 1991).

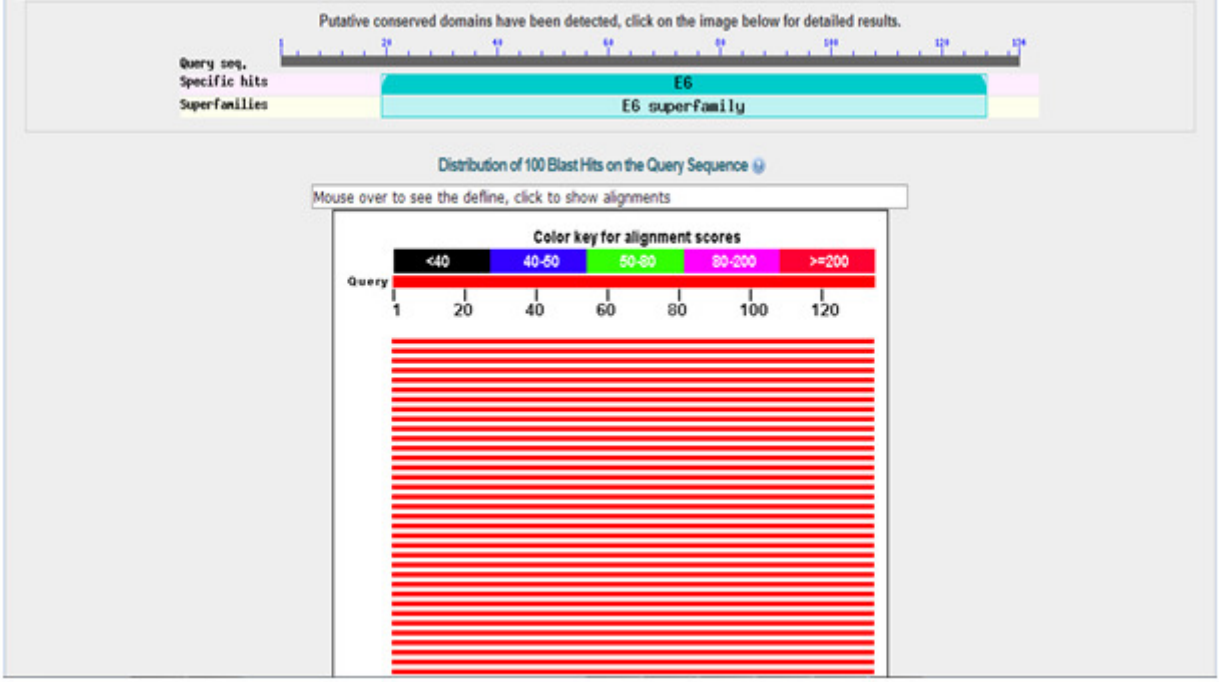


**Fig. 3 :** View result of BLAST.

Finally, the viral particles are aggregated in the nucleus, and the complete virions are released when the cornified layers of the epithelium are shed. The virions are shed in an environment with desquamated cells in the absence of lysis or necrosis and this further contributes to virus persistence because it avoids inflammation (Stanley, 2010).

Most females infected with a HPV type will not show proof of that same kind after six-twelve months. It is not known whether the HR-HPV can be reveal for periods like into these for LR-HPV. Several studies show same duration (Richardson *et al*, 2003), while others detect longer durations of infection for HR-HPV kinds (Franco *et al*, 1999; Ho *et al*, 1998).

## MATERIALS AND METHODS

The current study performed out by using varied tools, biological databases like PubMed, PubChem, PDB (Protein Data Bank) and software like gold Chemsketch 14.7.21.0.

### Basic Local Alignment Search Tool (Blast)

It is specifically designed to search nucleotide and protein database. It takes DNA or protein sequence and searches either DNA or protein databases for levels of identity that range from perfect matches to very low similarity. Using statistics, it returns back to the user what it finds, in the form of graphics or tables as well as, alignments.

### Secondary structure prediction

According to (URL: npsa-pbil.ibcp.fr/cgi-bin/secpred_gor4.pl). We got secondary structure prediction, as shown in next view result (Fig. 4-A & B).

### Functional analysis

Analyzing protein sequences with the ProtParam module

Before having docking step with gold. Protein sequences can be analyzed by several tools, but based on the ProtParam tools on the Expasy Proteomics Server.

### Phylogenetic analysis

Another tool is (Phylogenetic Analysis). We use it before docking, to know the relationships between organisms individuals (such as species, strains), as well, understanding of the evolutionary history for them.

### Protein binding site prediction:

Epitope prediction: 2FK4

We use IEDB Analysis Resource to determine 2FK4 as Epitope Prediction and use it with docking step.

### Antigenicity prediction

Use this step to determine the predicted antigenic peptides. The function of this program predicts those segments from within a protein sequence that are likely to be antigenic by eliciting an antibody response. While this step depends on use of Kolaskar and Tongaonkar (1990) method.

### Docking setup (using gold)

Molecular docking plays a crucial role in the design of arithmetic drugs. Docking predicts the preferred

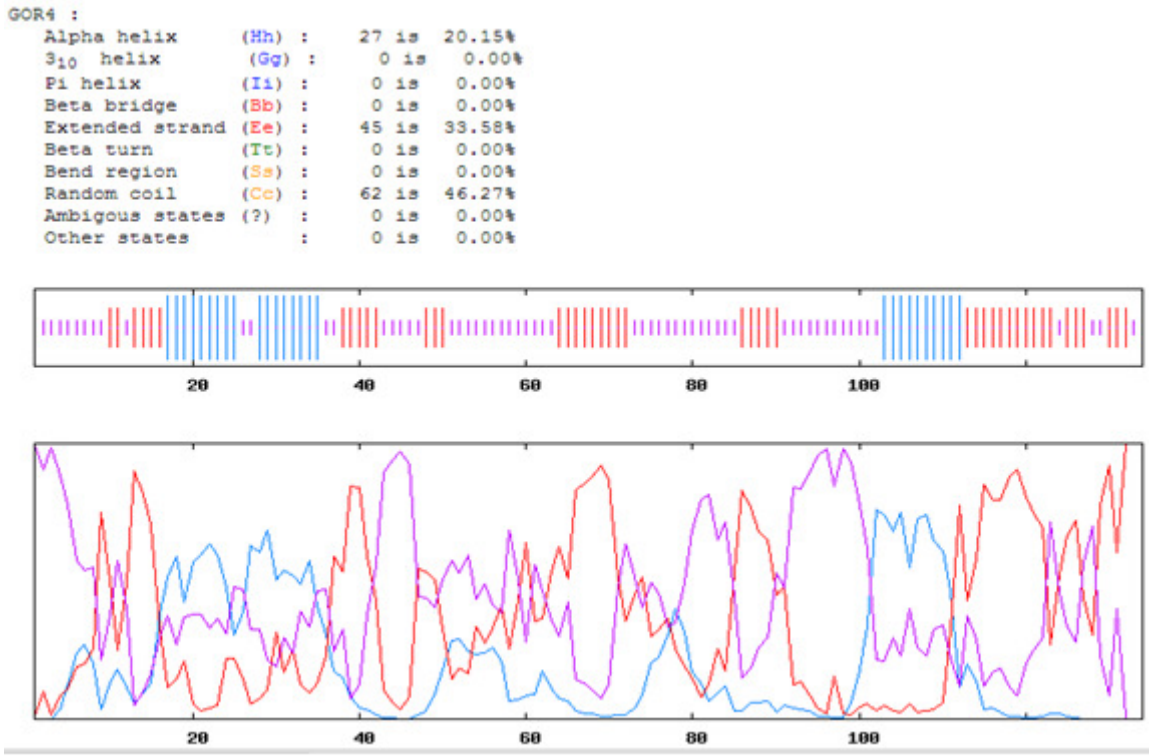


**Fig. 4 A:** View result of secondary structure prediction.

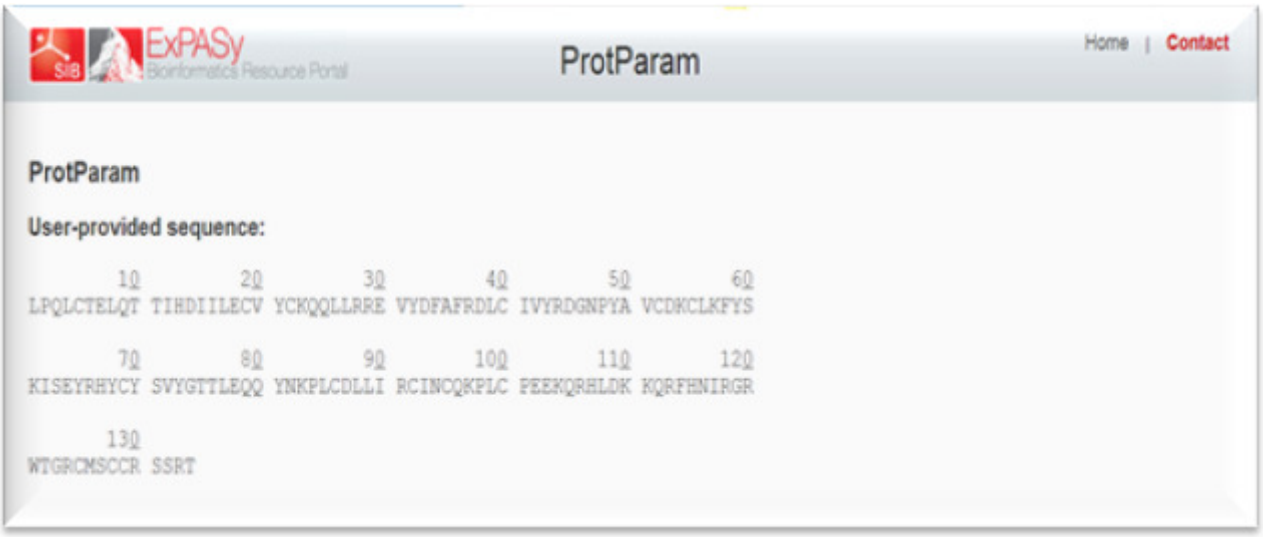


**Fig. 7:** Shows result of amino acids details of protein by ProtParam tool.

orientation of a lig and with the binding site on receptors.

## 1. Target

From RCSB and Protein Data Bank (PDB), we brought the data of HPV E6, E7 which carry with ID:

2FK4. Length: 134 amino acids. Molecular Weight: 16122.7 g/mol. Theoretical pI: 8.85. Molecular Formula: $C_{706}H_{1112}N_{204}O_{199}S_{15}$ (PubChem.ncbi) was used as target complex structure in the current study and apigenin drug which carries ID: 3DDN and save it within computer in pdb format. As other Target proteins are 2LJY, 2LJX.

## 2. Ligand selection

PubChem database is an online database contains many of chemical drugs (ligand) which provide the information and structure of chemicals. Many antiviral molecules were taken from the National Centre for Biotechnology Information (NCBI) PubChem compound database (URL: bi.nlm.nih.qov/) as ligand molecules. These molecules were downloaded in Structure Data File (SDF) format. Where selected for docking with HPV E6, E7 and Apigenin drug, adatiscetin, isorhamnetin, separately act as inhibitors.

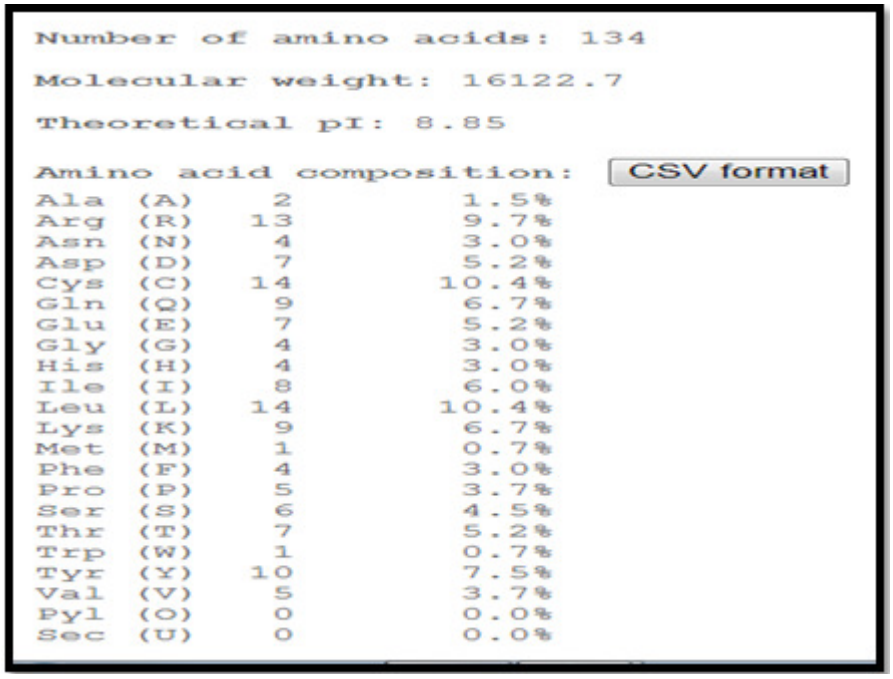


**Fig. 8 :** View of similarity in phylogenetic tree.

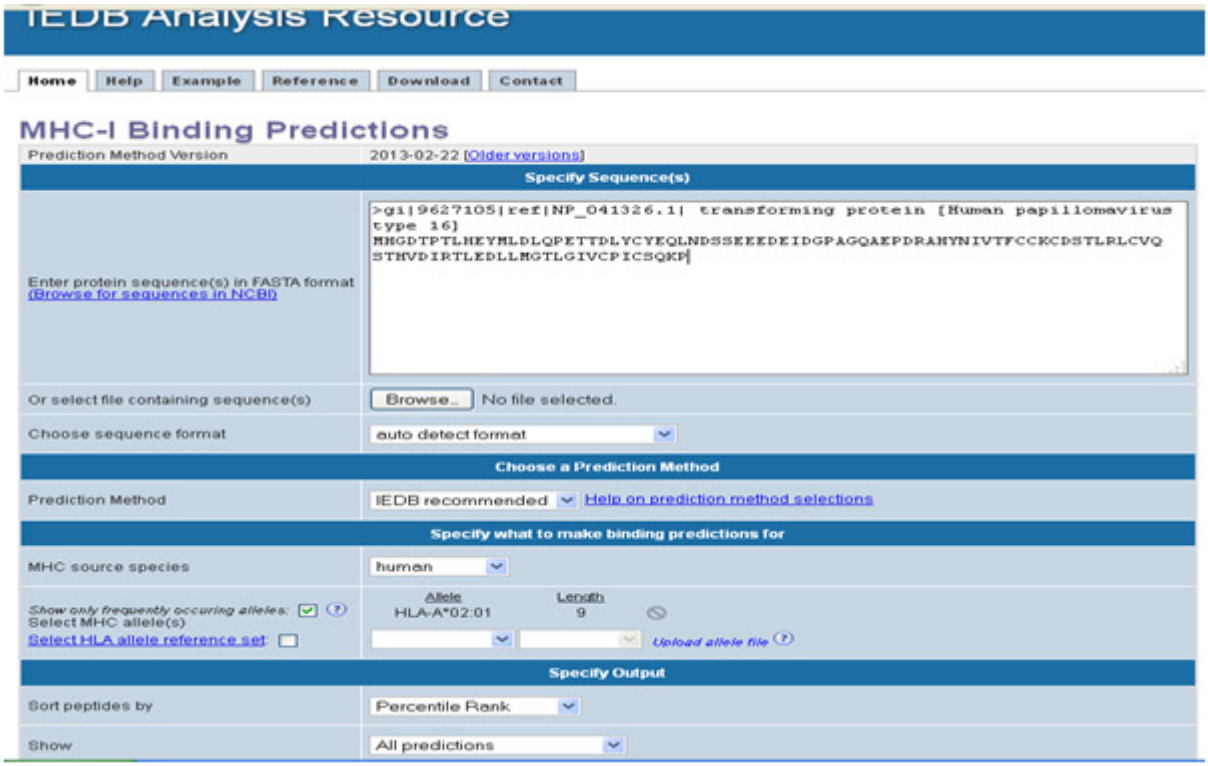


**Fig.**

## 3. Setting up GOLD Parameter

The protein molecule of E6, E7, apigenin drug, adatiscetin, isorhamnetin were uploaded into GOLD. The ligands were also uploaded. GOLD was run in a particular way such that a particular atom number was given from the identified active site. The output folder was specified. All the other fitness function parameters and the genetic algorithm parameters were kept in default mode. We used Pymol to view the GOLD output.

## RESULTS

As comparing HPV E6 & E7 target proteins by using in silico approaches Epitope predictions (EP), Antigenicity (Ag), Immunogenicity (IM) of target proteins and docked with different ligands among them, these results one of the best proteins gives the best result for, to find out best

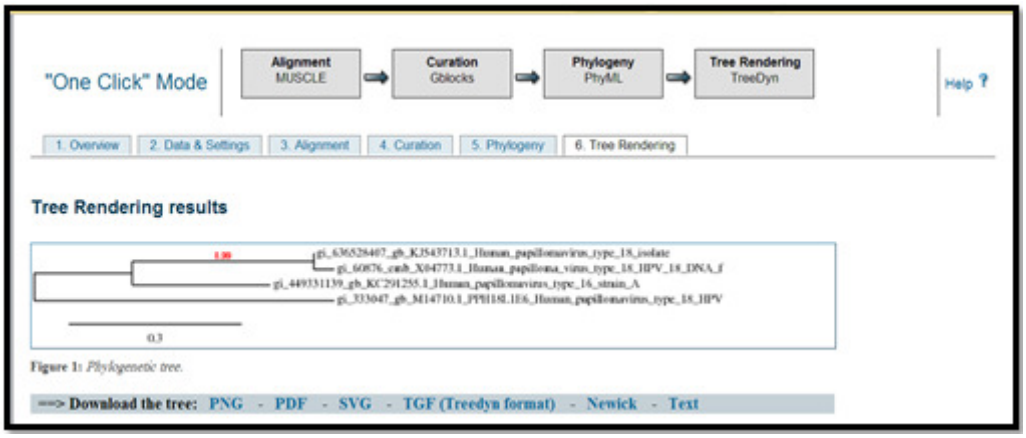


**Fig. 9:** Shows of Epitope Prediction determine to use at docking step.

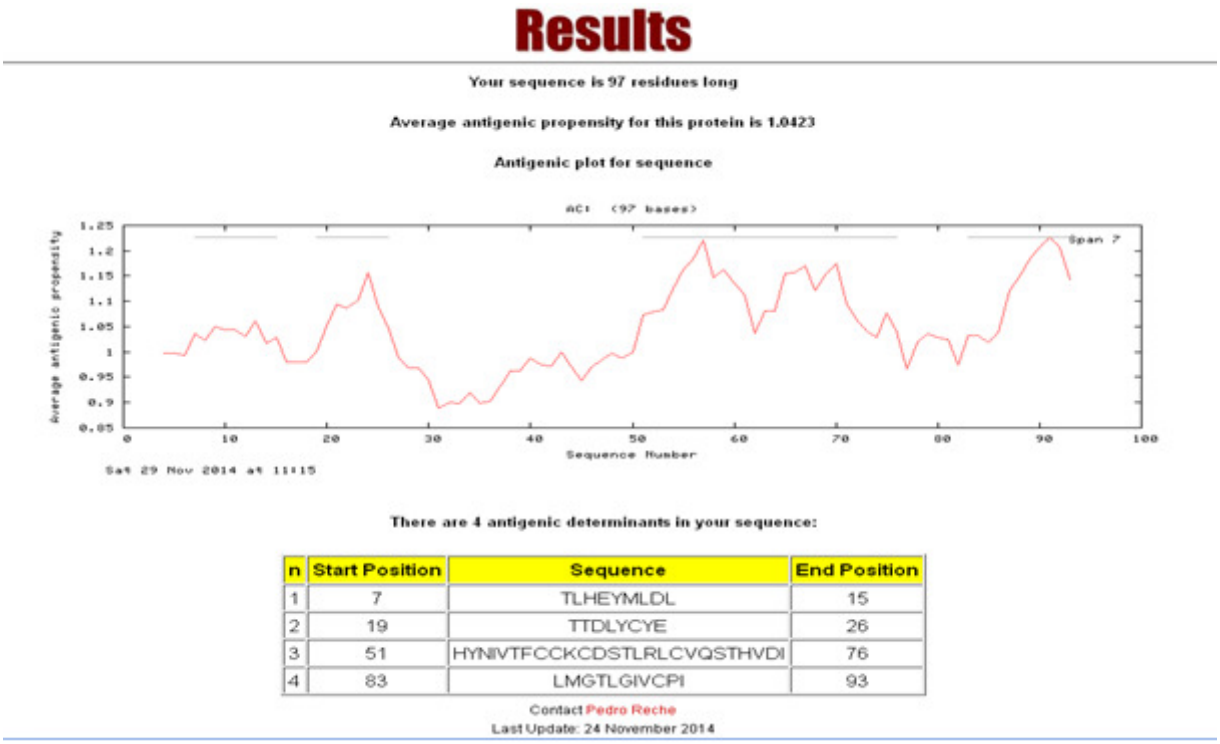


**Fig. 10 :** Results of Antigenicity prediction step.

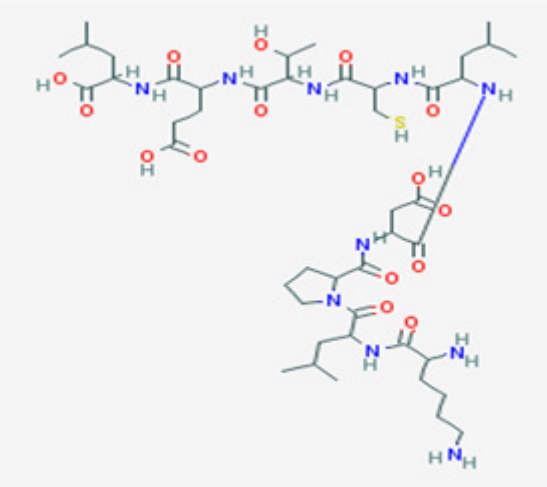

**Fig. 11 :** 2D structure of HPV E6.

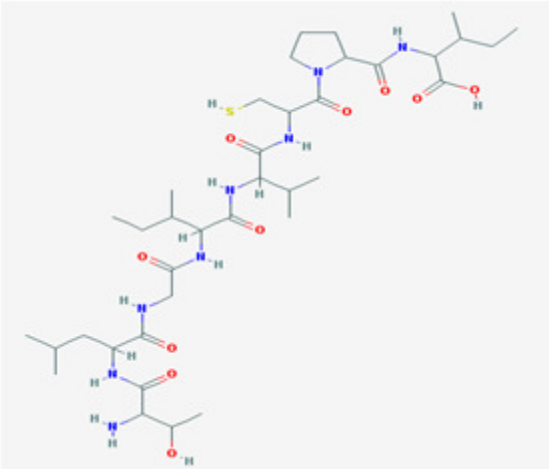

**Fig. 12 :** 2D structure of HPV E7.

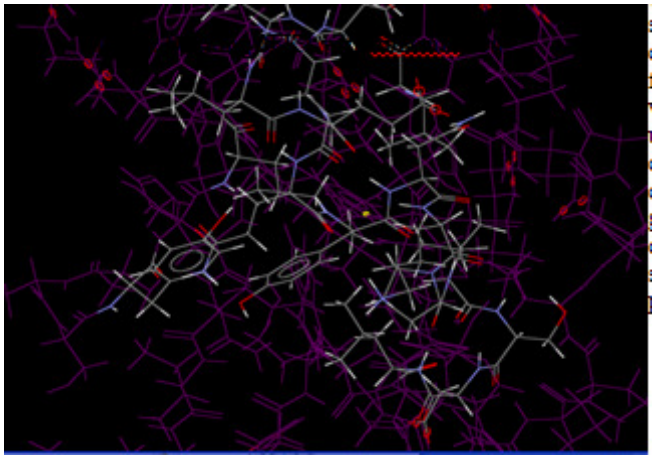

**Fig. 13 :** Docking 2FK4 with apigenin drug.

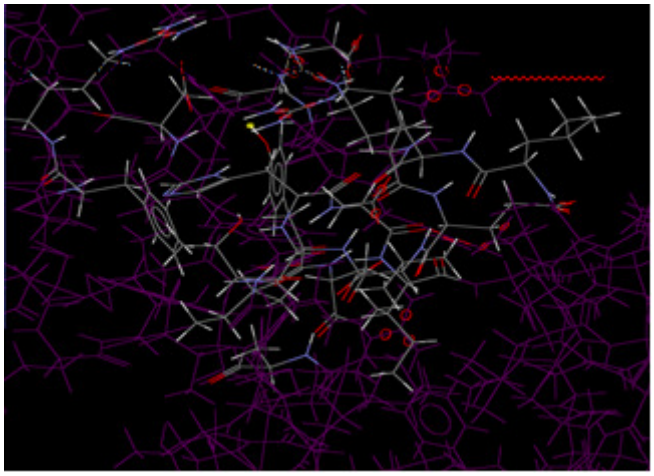

**Fig. 14 :** Docking interaction between 2LJY with adatiscetin.

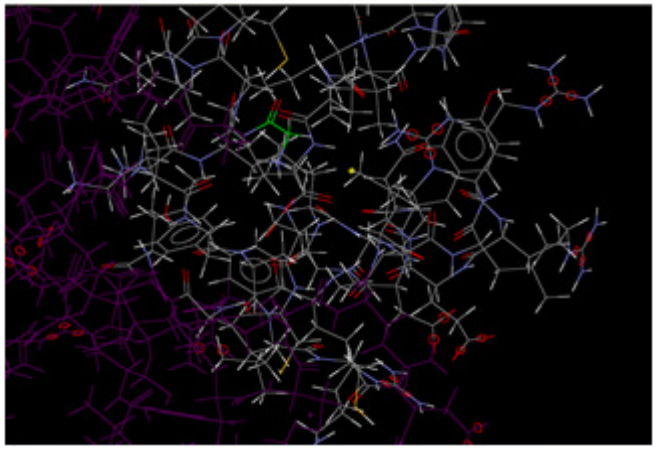

**Fig. 15 :** Docking Interaction between 2LJX with isorhamnetin.

target and ligands. This information will help to future advanced drug development for causative cancers.

Here, 2FK4, 2LJY, 2LJX are the target proteins for them results are respectively EP (low percentile ranks good binders – 0.4, ANN – 0.3, SMM – 0.4), Ag – is 1.0423 (propensity value), IM score 0.38722 and finally docking fitness score-72.58; EP (low percentile ranks good binders –2.2, ANN – 2.2, SMM – 1.8), Ag – is 1.0655 (propensity value), IM score 0.38898 and finally docking fitness score

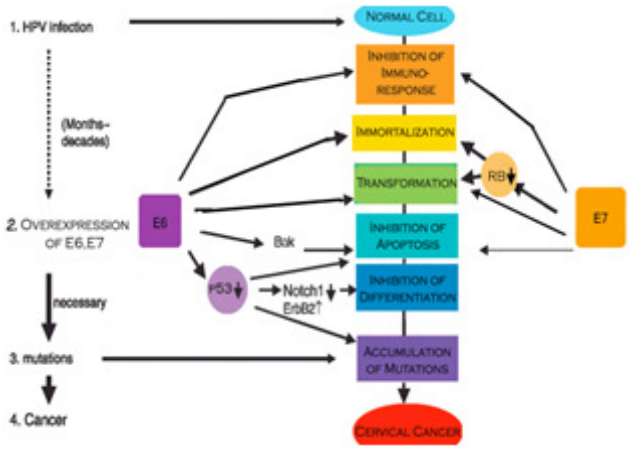


**Fig. 16 :** Mechanism of E6E7 in carcinogenesis of cervical cancer.

-95.52 ; EP ( low percentile ranks good binders – 2.2, ANN – 2.2, SMM – 1.8), Ag – is 1.0681 (propensity value), IM score 0.38898 and finally docking fitness score -180.65.

## DISCUSSION

Cervical cancer is one of the leading world causes of cancer morbidity and mortality in a woman, with more than 98% related to a human papillomavirus (HPV) infection origin. Infection with specific subtypes of HPV has been strongly implicated in cervical carcinogenesis. The identification and functional verification of host proteins associated with HPV E6 and E7 oncoproteins may provide useful information in understanding cervical carcinogenesis and the development of cervical cancer-specific markers. The advent of functional genomics and proteomics has provided hope of discovering novel biological markers for use in the screening, early diagnosis, prognostication and prediction of response to therapy.

## CONCLUSION

Advances in immunology, genomics and proteomics have accelerated our understanding of the genetic and cellular basis of many cancer types. Cervical cancer is a member of the virus-related neoplasms, with its initiation and promotion associated with persistent infection of oncogenic HPV.

Proteomics is now widely accepted as a useful tool in the development of molecular diagnosis and the identification of disease biomarkers in the post-genomic era. Proteomics also promises lower R&D costs, with the opportunities of new revenue streams through the identification of new drug targets in the treatment of various cancers. This review identifies the key technologies that will enable pharmaceutical companies to develop new niche products, improve drug attrition rates, increase the speed of clinical development and target

new drug markets.

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