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Neutralization of Hepatitis C through a Synthetic Peptide Potent Vaccine by Structural Designing of Disease Causing Strain Hepc1 (Isolate 1)

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Abstract: Hepatitis C virus (isolate 1) strain is a virus which has a genomic configuration of RNA by causing a disease in humans as Hepatitis C. Hep C is very dangerous and there is no vaccine yet discovered. This Hep C is passed through contaminated blood i.e. only blood is the mode of transmission for this virus which occurs due to sexual contact. This virus transcribes number of proteins which are responsible for various attributes it contains 3 structural and 7- non structural gene among which core protein E1, E2 and p7 are structural proteins and p23, p70, p8, p27, p56/58, p68 are non structural forms. By using all these proteins this Hep C virus causing all sorts of liver diseases, but still there is no specific vaccine for this disease. These resulting impairments of liver functions cause the disabilities associated with Hepatitis. The present study summarizes recent advances in understanding of biology of Hepatitis, clinical features of this disease and current diagnostic criteria and new approaches to treat the infection and immune mediated complications. The main objective of this project is to design the development of vaccine against hepatitis by Reverse Vaccinology Approach. Hepatitis c virus (isolate1) is the only viral strain that is causing liver chronic i.e. chronic disorders more globally with 4 different proteins. After screening all the proteins it was found out that the protein sequences with less identity was chosen. Antigen determinant was found out from particular protein sequence to find the epitope prediction through

various tools. Determined the structure and binding of MHC I to Antigen of the pathogen by Molecular Docking

Keywords: Hepatitis C, Reverse Vaccinology, Antigenic determinants, Molecular Docking

INTRODUCTION

Hepatitis C is a contagious liver disease that ranges in severity from a mild illness lasting a few weeks to serious, life long illness that attacks the liver[1]. It results from infection with the Hepatitis C virus (HCV), which is spread primarily through contact with the blood of an infected person. Hepatitis C can be either "acute" or "chronic." Acute Hepatitis C virus infection **i**s a short-term illness that occurs within the first 6 months after someone is exposed to the Hepatitis C virus[2]. For most people, acute infection leads to chronic infection.

Chronic Hepatitis C virus infection **i**s a long-term illness that occurs when the Hepatitis C virus remains in a person's body[3]. Hepatitis C virus infection can last a lifetime and lead to serious liver problems, including cirrhosis (scarring of the liver) or liver cancer.

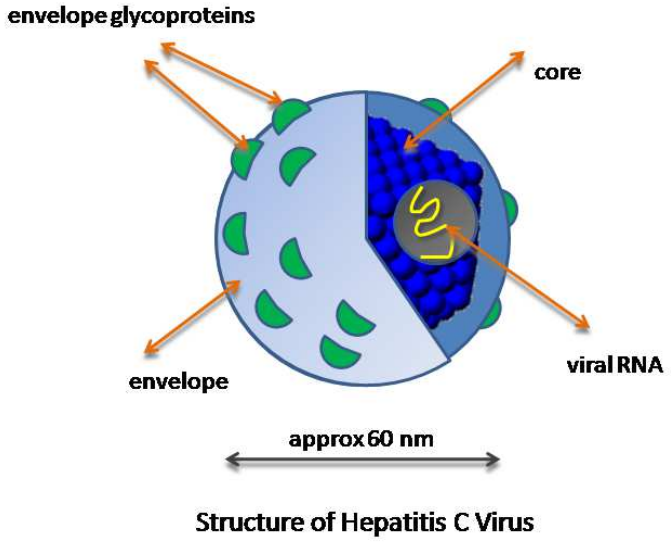

Fig 1: General Structure of Hepatitis C virus

In 2009, there were an estimated 16,000 acute Hepatitis C virus infections reported in the United States[4]. An estimated 3.2 million persons in the United States have chronic Hepatitis C virus infection. Most people do not know they are infected because they don't look or feel sick. Hepatitis C is usually spread when blood from a person infected with the Hepatitis C virus enters the body of someone who is not infected[5]. Today, most people become infected with the Hepatitis C virus by sharing needles or other equipment to inject drugs. Before 1992, when widespread screening of the blood supply began in the United States, Hepatitis C was also commonly spread through blood transfusions and organ transplants[6]. People can become infected with the Hepatitis C virus during such activities as

- Sharing needles, syringes, or other equipment to inject drugs

- Needle stick injuries in health care settings
- Being born to a mother who has Hepatitis C

Less commonly, a person can also get Hepatitis C virus infection through

- Sharing personal care items that may have come in contact with another person's blood, such as razors or toothbrushes

Having sexual contact with a person infected with the Hepatitis C virus Hepatitis C is an sexual transmitted disease, but the risk of transmission from sexual contact is believed to be low[7]. The risk increases for those who have multiple sex partners, have a sexually transmitted disease, engage in rough sex, or are infected with HIV. More research is needed to better understand how and when Hepatitis C can be spread through sexual contact. The Hepatitis C virus can survive outside the body at room temperature, on environmental surfaces, for at least 16 hours but no longer than 4 days[8]. Hepatitis C virus is not spread by sharing eating utensils, breastfeeding, hugging, kissing, holding hands, coughing, or sneezing. It is also not spread through food or water. Approximately 70%–80% of people with acute Hepatitis C do not have any symptoms[9]. Some people, however, can have mild to severe symptoms soon after being infected, including Fever, Fatigue, Nausea , Abdominal pain Dark urine, Clay-coloured bowel movements , Joint pain, Jaundice (yellow colour in the skin or eyes) Most people with chronic Hepatitis C do not have any symptoms. However, if a person has been infected for many years, his or her liver may be damaged. In many cases, there are no symptoms of the disease until liver problems have developed[10]. In persons without symptoms, Hepatitis C is often detected during routine blood tests to measure liver function and liver enzyme (protein produced by the liver) level. Chronic Hepatitis C is a serious disease that can result in long-term health problems, including liver damage, liver failure, liver cancer, or even death. It is the leading cause of cirrhosis and liver cancer and the most common reason for liver transplantation in the United States. Approximately 15,000 people die every year from Hepatitis C related liver disease[11]. This Hepatitis C virus mainly occurs in 5 different types of viruses modes Hep A, Hep B, Hep C, Hep D, Hep E. Where each and every one vary by their mode of transmission and there way of causing severity of the disease. Among all these types of viruses Hep c is dangerous and there is no vaccine yet discovered. Hepatitis C was first detected in 1989 using molecular biology techniques after extensive testing of serum from experimentally infected animals[12]. It was later characterized to be an RNA virus that belongs to the Flaviviridae family and genus Hepa c virus. Ever since its discovery it became clear that this virus was the major cause of acute hepatitis after a blood transfusion that was neither related to Hepatitis A nor to hepatitis B[13]. The Hepatitis C virus genome is comprised of a single stranded positive-sense RNA with a single opening reading frame of 9.6 kb in length encoding for a single poly protein precursor of approximately 3000 residues flanked by un translated regions (UTRs) at both ends. The precursor is cleaved into at least 10 different proteins: the structural proteins Core, E1, E2 and p7, as well as the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B. An important feature of the HCV genome is its high degree of genetic variability. Reverse vaccinology**:** Although successful in many cases, this approach is time-consuming and fails when the pathogens cannot be cultivated in vitro, or when the most abundant antigens are variable in sequence[14]. Now genomic approaches allow prediction of all antigens, independent of their abundance and immunogenicity during infection, without the need to grow the pathogen in vitro. This allows vaccine development using non-conventional antigens and exploiting non-conventional arms of the immune system. Many vaccines impossible to develop so far will become a reality[15]. Since the process of vaccine discovery starts in silico using the genetic information rather than the pathogen itself, this novel process can be named reverse vaccinology.

MATERIALS AND METHODS

Materials used for this study is NCBI taxonomy browser for identifying the strain, SDSC biological work bench for The biology workbench is a point and click interface for searching protein and nucleic acid sequence database and for analysis sequence data, Immunomed for This program predict those segments from within a protein sequence that are likely to be a antigenic eliciting as antibody response, Emboss Antigenic for Finds antigenic sites in proteins, MAPPP for This will predict possible antigenic peptides to be processed and finally presented on cell surface, Bimas for Finds Antigenic sites in the protein, HLA for Finds antigenic sites in the protein, SOPMA for Secondary structure analysis, HNN for Secondary structure analysis, HH- Pred for Necessary for PDB ID identification, CPH model for Necessary for PDB ID identification, Geno3D for Necessary for PDB ID identification, PDB(Protein data bank) for MHC molecule prediction, DISCOVERY STUDIO for It is used for energy minimization, creating a molecule and docking(CDOCKER) , PAProc for It is the predicted tool for cleavages by human and yeast 20S proteosomes, based on experimental cleavage data, BLAST for Protein sequence comparison with different model animals tool, SDSC(CLUSTAL W) for Phylogeny analysis.

RESULTS

NCBI TAXONOMY BROWSER

>Protein Sequence 1

MSTNPKPQKKNKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGR
RQPIPKARRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSPRGSRPSWGPTDPRRRSRNLGKV
IDTLTCGFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCT
VPASAYQVRNSTGLYHVTNDCPNSSIVYEAADAILHTPGCVPCVREGNASRCWVAMTPTVA
TRDGKLPATQLRRHIDLLVGSATLCSALYVGDLCGSVFLVGQLFTFSPRRHWTTQGCNCSIYP
GHITGHRMAWDMMMNWSPTTALVMAQLLRIPQAILDMIAGAHWGVLAGIAYFSMVGNWA
KVLVVLLLFAGVDAETHVTGGSAGHTVSGFVSLLAPGAKQNVQLINTNGSWHLNSTALNCN
DSLNTGWLAGLFYHHKFNSSGCPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPP
KPCGIVPAKSVCGPVYCFTPSPVVVGTTDRSGAPTYSWGENDTDVFVLNNTRPPLGNWFGCT
WMNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSGPWITPRCLVDYPYR
LWHYPCTINYTIFKIRMYVGGVEHRLEAACNWTRGERCDLEDRDRSELSPLLLTTTQWQVLP
CSFTTLPALSTGLIHLHQNIVDVQYLYGVGSSIASWAIKWEYVVLLFLLLADARVCSCLWMM
LLISQAEAALENLVILNAASLAGTHGLVSFLVFFCFAWYLKGKWVPGAVYTFYGMWPLLLL
LLALPQRAYALDTEVAASCGGVVLVGLMALTLSPYYKRYISWCLWWLQYFLTRVEAQLHV
WIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAVFGPLWILQASLLKVPYFVRVQGLLRFC
ALARKMIGGHYVQMVIIKLGALTGTYVYNHLTPLRDWAHNGLRDLAVAVEPVVFSQMETK
LITWGADTAACGDIINGLPVSARRGREILLGPADGMVSKGWRLLAPITAYAQQTRGLLGCIIT
SLTGRDKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGPVIQMYTNVDQ
DLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLL
CPAGHAVGIFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPT
GSGKSTKVPAAYAAQGYKVLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYST
YGKFLADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVP
HPNIEEVALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKC

>Protein sequence 2

>gi|58177082|pdb|1W3C|A Chain A, Crystal Structure Of The Hepatitis C Virus Ns3 Protease In Complex With A Peptidomimetic Inhibitor

APITAYSQQTRGLLGCIITSLTGRDKNQVDGEVQVLSTATQSFLATCVNGVCWTVYHGAGSK
TLAGPKGPITQMYTNVDQDLVGWPAPPGARSMTPCTCGSSDLYLVTRHADVIPVRRRGDSR
GSLLSPRPVSYLKGSSGGPLLCPSGHVVGIFRAAVCTRGVAKAVDFIPVESMETTMRSPVFTD
N

>Protein sequence 3

>gi|58177083|pdb|1W3C|B Chain B, Crystal Structure of the Hepatitis C Virus Ns3 Protease in Complex with APeptidomimetic Inhibitor

APITAYSQQTRGLLGCIITSLTGRDKNQVDGEVQVLSTATQSFLATCVNGVCWTVYHGAGSK
TLAGPKGPITQMYTNVDQDLVGWPAPPGARSMTPCTCGSSDLYLVTRHADVIPVRRRGDSR
GSLLSPRPVSYLKGSSGGPLLCPSGHVVGIFRAAVCTRGVAKAVDFIPVESMETTMRSPVFTD
N

EPITOPE RESULTS

VLA SEQUENCE-4

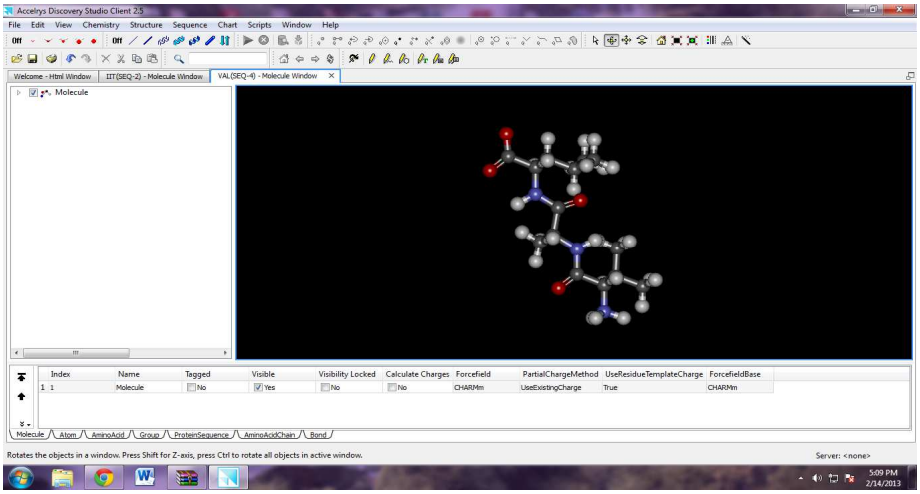

Fig.2: Epitope prediction results from Accerly discovery studio for VLA sequence 4

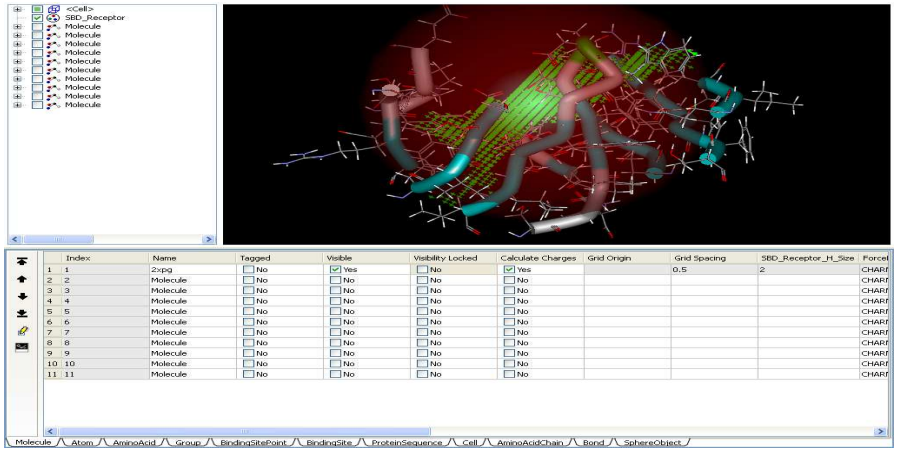

Fig 3: Spherical MHC molecule before the removal of surrounding water molecules

>Protein sequence 4

>gi|68565345|sp|P0C044.2|F_HCV1 RecName: Full=F protein; AltName: Full=Alternate reading frame protein/F-protein; Short=ARFP/F; AltName:Full=Frameshifted protein; AltName:Full=p16;AltName: Full=p17

MSTNPKPQKKKTNVTPTVAHRTSSSRVAVRSLVEFTCCRAGALDWVCARRERLPSGRNLEV DVSLSPRLVGPRAGPGLSPGTLGPSMAMRAAGGRDGSCLPVALGLAGAPQTPGVGRAIWVR SSIPLRAASPTSWGTYRSSAPLLEALPGPWRMASGFWKTA

FOR SEQUENCE-4

IMMUNOMED	EMBOSS	BIMAS	HLA	MAPPP
SSSRVAVRSLVEFTCC RA	SSRVAVRSLVEFTCCR AG	SCLPVALGL	SCLPVALGL	SCLPVALGL
ALDWVCAR	LDWVCARR	SCLPVALGL	SCLPVALGL	SCLPVALGL
LEVDVSLSPRLVGP	EVDVSLSPRLVGPR	SCLPVALGL	SCLPVALGL	SCLPVALGL
DGSCLPVALGLAGA	GSCLPVALGLAGAP	SCLPVALGL	SCLPVALGL	SCLPVALGL

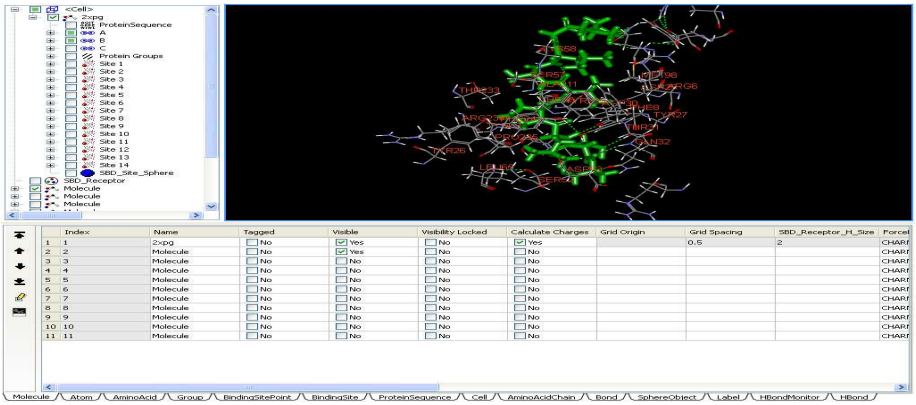

Fig 4: Binding of Epitope (ligand) to MHC molecule (Receptor) after Docking

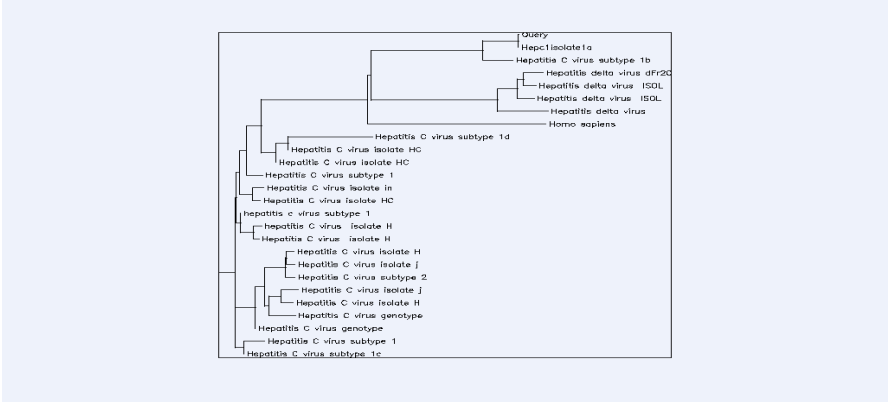

Fig 5: Phylogenetic relation of 15 different species with query (fourth protein sequence)

CONCLUSION

Hepatitis C is a viral disease that occurs due to Hepatitis C virus strains causes chronic disease like cirrhosis for liver. This usually occurs due to blood transfusion during sexual contact. A supposed vaccine was designed against Hepatitis C viral strain and apart from its analogues protein of its

antigen were also determined and this in turn identify the similarity between the protein sequence of Hep C 1 (isolate 1) with other species From this study, it was concluded that, a type of MHC molecule on which the Antigen determinants binds to form Antigen-Antibody complex. By using several tools like SDSC biology work bench, Immunomed, Emboss Antigenic...etc. the proteosomal sequences were identified. The qualifying vaccine was taken for docking. From docking results the vaccine which has having minimum CDOCKER energy is selected as potent and approved vaccine for Hepatitis.

REFERENCES

1. D.Lavanchy, Evolving epidermiology of Hepatitis C virus , *Clinical microbiology journal 2011 ; 17:107-15*
2. T.J. Liang,B. Rehermann, L.B.Seeff, J.H.Hoofnagle , Pathogenesis natural history treatment and prevention of Hepatitis C , *Ann intern Med 2000; 132:296-305*
3. M.J.Alter,D., Kruszon-moran, D. Nainan OV *et al* the prevalence of Hepatitis C virus infection in the united states,1988 through 1994 N eng *J Med 1999 ; 341:556-62*
4. Department of health and human services, combating the silent epidemic of viral hepatitis action plan for the prevention, *care and treatment of viral hepatitis 2011 available at http://www.hhs.gov/ash.*
5. W.O.Osburn, B.E. fisher, K.A. Dowd, *et al.*, spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection, *Gastroenterology 2010 ; 138:315-24*
6. H.Dahari, S.M. Feinstone, M.E. Major, metaanalysis of hepatitis C virus vaccine efficacy in chimpanzees indicates an importance for structural proteins *Gastroenterology 2010;139:965-74*
7. *M.* Puig, K. Mihalik,J.C. Tilton, *et al* CD4+ immune escape and subsequent T-cel failure following chimpanzee immunizatiogainst hepatitis C virus *Hepatology 2006 ; 44:736-45l*
8. A. Folgori, S. Capone,L. Ruggeri, *et al.* A T-cell HCV vaccine eliciting effective immunity against heterlogous virus challenge in chimpanzees *nat med 2006; 12:190-7*
9. I.Zubkova, Y.H. Choi, Chang *et al* T-cell vaccine that elicit effective immune responses against HCV in chimpanzees may creat greater immune pressure for viral mutation *vaccine 2009; 27:2594-602*
10. S.E.Frey,M. Houghton, Coates S *et al* safety and immunogenicity of HCv E1E2 vaccine adjuvanted with MF59 administered to healthy adult *vaccine 2010;28:6367-73*
11. Z.Stamataki, S. Coates ,S. Abrignano, M. Houghton,J.A. Mc keating, immunization of human volunteers with Hepatitis C virus envelop glycoprptein elicits antibodies that cross-neutralizes heterologous virus strain *J infect Dis 2011;204:811-3*
12. 12.A. Chmielewska, M. Naddeo , *et al* Enhancing T cell reponses and cross neutralization antibodies against HCV envelope by combining adenovirus and protein. *18th international symposium on hepatitis C virus, seattle, Washington 8-12 september 2011 (abstract 06.04)*
13. A.Harari, P.A. Bart,W. Stohr , *et al* An HIV -1 clade CDNA prime, NYVAC boost Vaccine regime induces reliable, polyfunctional & long-lasting Tcell responses *J Exp Med 2008;205:63-77*
14. A.Tungol, K. Rademacher, J.A. Schafer , Formulating management of the protease inhibitors boceprevir and telaprevir for chronic hepatitis C virus.*J manag care pharm 2011;17:685-94*
15. S. Yutani, A. Yamada, K. Yoshida, *et al* Phase 1 clinical study of a personalized peptide vaccination for patitents infected with hepatitis C virus (HCV)1b who failed to respond to interferon-based therapy *vaccine 2007;25:7429-35*

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