Int J Pharm Bio Sci 2014 Oct; 5(4): (B) 1212 - 1222

ResearchArticle IMMUNOBIOLOGY

International Journal of Pharma and Bio Sciences ISSN 0975-6299

# PREDICTION OF HLA-DRB1*15 RESTRICTED $CD_4$+ EPITOPES OF INDIAN HIV-1 GAG PROTEIN USING INSILICO APPROACH

**JEMMY CHRISTY.H[1*] AND ALEX ANAND.D[2]**

*Department of Bioinformatics[1], Department of Biomedical Engineering[2] Sathyabama University, Chennai-119, India.*

## ABSTRACT

Human immunodeficiency virus (HIV) a retrovirus that belongs to the Lentiviridae family, is the causative agent for Acquired Immuno Deficiency Syndrome. Certain “protective” HLA alleles play significant role low viraemia condition like HLA-DRB1*15 allele. An Insilco analysis was adopted to identify Gag epitopes vaccine candidates namely P1-P6 (GLNKIVRMYSPTSIL,LGLNKIVRMYSPTSI,LNKIVRMYSPTSILD,NKIVRMYSPTSILDI,IVRMYSPTSILDIKQ,KIVRMYSPTSILDI) restricted to HLA-DRB1*15 allele would aid significant CD4+ T cell immune response against HIV infection and population coverage among Indian population assessed. Three dimensional structure of epitopes P1-P6 modeled using PEPFOLD, and were tested for their binding affinity towards HLA binding groove using GRAMMX based docking studies resulted with conventional hydrogen bonding,pi-donor,pi-sulfur,carbon hydrogen bonding ,pi-alkyl interaction. Thus the interaction between the screened P1-P6 epitopes exhibiting stability would aid immune response. This computational study of a Gag epitope-based peptide vaccine design for HIV would be helpful in determining novel peptide antigen targets in GAG proteins which need to be validated in vitro and in vivo experiments.

**KEYWORDS:** **HIV Vaccine, HLA-DRB1*15, Gag epitope, CD4+ T cell response, HLA-Epitope, Affinity.**

**JEMMY CHRISTY.H**
**Department of Bioinformatics Sathyabama University, Chennai-119, India.**

# INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) a major health concern is caused by *Human immunodeficiency virus* (HIV), a retrovirus that belongs to the Lentiviridae family. Human immunodeficiency virus-1 (HIV-1) has infected more than 75 million people and caused nearly 36 million deaths worldwide[2]. According to National AIDS control organization recent report (2013-14) India has the third highest number of estimated people living with HIV in the world and it is estimated that 20.89 lakh people living with HIV/AIDS in India[3] . Highly active anti retro viral therapy (HAART) based on combination antiretroviral drugs are used to treat individuals with HIV infection each with its own side effects[4]. HAART therapy reduces the viral load and leading to a declination in morbidity and mortality of HIV-infected individuals, but cannot eradicate the virus[4].So there is a prospect for HIV vaccine has long been a key area for research and numerous resources have been directed for HIV vaccines development[5-10]. Complex architecture seen in the HIV-1 genome comprise of three functional groups of genes like structural genes ( Gag, Env ,Pol) ,regulatory genes (Tat,Rev) and accessory genes (Vpu,Vpr,Vif,Nef )[11]. Decades of HIV research reveals a fact that designing of vaccine for globally HIV infected people is not possible, since genetic diversity of HIV and clade /subtype differences are the major obstacles[12, 13]. Three distinct groups: M (Major), O (Outliers), and N (non-M and non-O) of HIV-1 circulating in a global level among M is the most predominant group of HIV-1 around the world , Within the M group there are nine subtypes: A–D, F–H, J, and K[14].Commonly known clade HIV-1C responsible for the burden of AIDS globally[15],So our study restricted to analysis to HIV-1 subtype C-Gag specific $CD4^+$epitopes. Hypermutation capability of HIV and HLA polymorphism are the keyfactors for designing a vaccine for HIV,so conservancy of Gag sequences among Indian population and their restriction to HLA alleles need to be studied[16,17]. Several class I HLA alleles like HLA-A02, HLA-A11, HLAB27, HLA-B*2705, HLA-B51, HLA-B*5701 have been reported to be associated with resistance or slow progression to HIV/AIDS[18-27]. But heterodimeric HLA class II restricted $CD4^+$ T cell responses to HIV disease outcome is poorly defined, recent studies revealed that on DRB1 allele expression in HIV among HIV controllers[28,29].Our study is based on highly polymorphic DRB1*1501-04 alleles affinity for Gag based CD4+ epitopes. Significant role of HLA on the outcome of disease would be useful in rational design of a HIV vaccine. Computational epitope prediction methods based on various algorithms are playing promising role in screening of and selection of CD4+ T cell Gag epitopes retricted to DRB1*1501-04 alleles which are highly expressed allele among south Indian population as well as associated withlow viraemia condition in HIV infected individuals.

# METHODOLOGY

### *Retrieval of Gag amino acid sequence and conservancy analysis*

HIV Sequence database[30] was used to retrieve the Indian HIV patients sequences (1991-2012) their Accession No, Patient code,Risk Factor ,Sampling Year were observed to assess the redundancy among the retrieved gag sequence. Conservancy of gag sequence of Indian sample analysed by constructing multiple sequence alignment using ClustalW [31] tool of EBI server[32] .From the clustalW the conservancy score for each amino acid position in gag sequence was obtained.

### *CTL epitope prediction, Population Coverage assessment*

CTL epitopes for Gag sequence was predicted using Immune Epitope Database (IEDB)[33] based on binding affinity between HLA-DRB1*1501-04 allele types and Gag epitopes. Currently IEDB prediction includes Consensus method[34], combinatorial library[34], NN-align (netMHCII-2.2)[35], SMM-align (netMHCII-1.1)[36], Sturniolo[37], and NetMHCIIpan[38] modules. The predicted output is given in units of $IC_{50}$nM for combinatorial library and SMM_align. Therefore a lower number indicates higher affinity. As a

rough guideline, peptides with $IC_{50}$values <50 nM are considered high affinity, <500 nM intermediate affinity and <5000 nM low affinity. The predicted result for Sturniolo is given as raw score. Higher score indicates higher affinity. For each peptide, a percentile rank for each of the three methods (combinatorial library, SMM_align and Sturniolo) is generated by comparing the peptide's score against the scores of five million random 15 mers selected from SWISSPROT database. A small numbered percentile rank indicates high affinity. The median percentile rank of the three methods was then used to generate the rank for consensus method. NetMHCIIpan method is used when Consensus and other methods such as SMM_align, NN_align,COMBLIB and/or Sturniolo are not available for a particular allele. However, if only one or two of these methods are available, NetMHCIIpan is used as second or third method.Low percentile ranked epitopes were screened and assessed for population coverage among Indian population.Population coverage of the conserved gag epitopes with the corresponding HLA-DRB1*1501-04 alleles were analyzed based on population coverage analysis tool of IEDB[39] depending on allelefrequencies.net database [40] a huge population dataset on the web.

### *Prediction of the 3D Structures of the Predicted Epitope and HLA-DRB1*15 Allele Structure retrieval*

Low percentile ranked top listed epitopes from IEDB results were screened and selected epitopes 3D structures were modeled using PEP-FOLD[41], a de novo based approach for peptide structure server accessible via RPBS Mobyle Portal to analyze the interactions with HLA-DRB1 *1501-14 alleles . Top listed models provided by the server were chosen for the docking study based on avg, gdt, max, q, tm the scores. The 3D structure of HLA-DRB1*15 allele was retrieved from PDB [42] with the accession number of 1BX2.

### *HLA-Peptide Interaction analysis*

To analyse the interaction between HLA-DRB1 allele molecules and Gag epitopes, a docking study was performed using GRAMM-X[43] which based on rigid body search with the assistance of Fast Fourier Transform (FFT) correlation and its simplified geomentry uses shape complementarity and hydrophobicity characters in the scoring function, but for Gag epitope and HLA docking GRAMM-X uses Fast Fourier Transformation methodology with smoothed Lennard-Jones potential implementation with refinement stage and knowledge-based scoring, thus resulting in better peptide-protein interaction. GRAMM-X output includes ten models ranked as the most probable prediction candidate based on the scoring function retrieved as a PDB file and finally the docked interaction between HLA and epitopes were visualized using DS visualizer 4.0[44].

## RESULTS AND DISCUSSION

### *Retrieval of Gag amino acid sequence and conservancy analysis*

Gag protein sequences of Indian patients were retrieved from HIV sequence database and sampling year and patient codes were assessed to limit the redundancy in the gag sequence data set and their conserved segments were assessed using clustal W.

### *CD4+ CTL Gag epitope and population Coverage assessment*

CTL epitopes of Gag protein restricted to HLA-DRB1*15 allele were predicted using Immune Epitope Database prediction server ,among 1964 resulted epitopes based $IC_{50}$ value, percentile rank and scores we selected total of 6 epitopes namely p1(GLNKIVRMYSPTSIL), p2(LGLNKIVRMYSPTSI),p3(LNKIVRMYSPTSILD),p4(NKIVRMYSPTSILDI),p5( IVRMYSPTSILDIKQ),p6(KIVRMYSPTSILDIK) as promiscuous epitopes of Gag protein and listed in Table1

**Table 1**
***Gag $CD_4$+ epitopes and prediction score***

| Allele | Peptide | Netmhcii $IC_{50}$ | Sturniolo Score | Smm_Align $IC_{50}$ | Nn_Align $IC_{50}$ |
|---|---|---|---|---|---|
| HLA-DRB1*15:01-04 | GLNKIVRMYSPTSIL | 14.29 | 6.8 | 30 | 3.2 |
| | NKIVRMYSPTSILDI | 19.98 | 6.8 | 32 | 3.5 |
| | IVRMYSPTSILDIKQ | 22.22 | 6.8 | 33 | 3.7 |
| | LGLNKIVRMYSPTSI | 22.16 | 6.8 | 33 | 3.6 |
| | KIVRMYSPTSILDIK | 28.08 | 6.8 | 37 | 4.9 |
| | LNKIVRMYSPTSILD | 24.56 | 6.8 | 31 | 4.9 |

***Epitope conservancy analysis of screened CTL epitopes***

Frequency of human MHC-HLA alleles differ among different ethinicities ,an ideal vaccine candidate from a pool of epitopes is selected based on their binding affinity towards the restriced HLA-DRB1*15 allele. The IEDB conservancy analysis tool analyzed the conservancy of the predicted Gag epitopes, which are shown in Table 2.

**Table 2**
***Population Coverage Calculation Result***

| Population / Area | Class II | | |
|---|---|---|---|
| | Coverage | Average hit | PC90 |
| India | 30.52% | 1.93 | 0.86 |
| Average (Standard deviation) | 30.52% (0.00%) | 1.93 (0.00) | 0.86 (0.00) |

***Modelling of 3D Structures of Epitopes using PEP-FOLD***

Low percentile ranked top listed epitopes Table.1 modeled using PEP-FOLD,and out of 5 proposed peptide models of PEP-FOLD server we selected the top listed peptide model p1-p6 epitopes **(Figure .1)** and their avg, gdt, max, q, tm the scores were recorded.Experimentally resolved 3D structure of HLA-DRB1*15 allele was retrieved from PDB with the accession number of 1BX2.

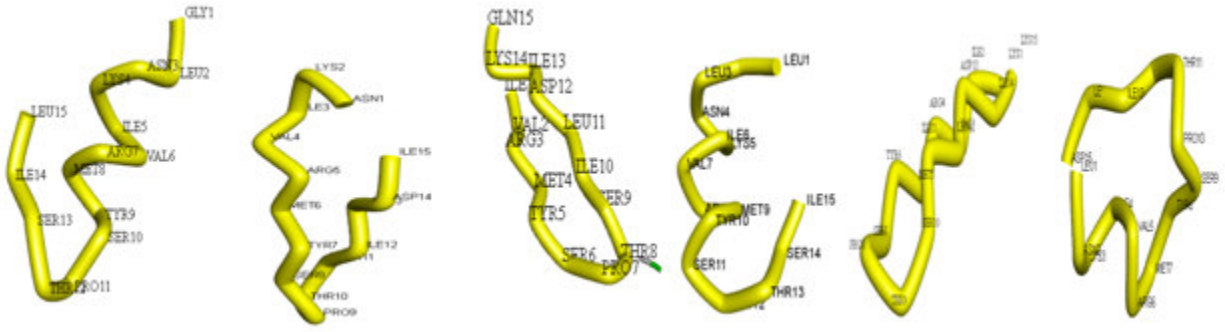


**Figure 1**
***3Dimensional structures of Gag Epitopes p1, p2, p3, p4, p5, p6***

***Molecular Docking of HLA-Epitope Interaction***

Using GRAMM-X tool binding models of predicted epitopes to their respective HLA-DRB1*15 molecules were generated and listed in (Figures2-7) .Docking analysis used to evaluate various conditions like whether the peptide is docked into the peptide binding groove or not, interacting bond distance, kind of interaction and anchoring residues of epitopes involvement in HLA-DRB1*15 allele interaction.The predicted epitope namely p1-p6 were bound within the binding groove of the HLA-DR1B*15 and their docking interaction were visualized with the DS visualizer 4.0 shown in Figure 3.Interaction between the HLA and epitopes included conventional Hydrogen bonds along with Pi-sulfur ,carbon hydrogen bonds ,pi donor hydrogen bond,salt bridges also stabilize the binding affinity of Gag epitopes on HLA-DRB1*15 allele binding groove their by it would confer immunity against HIV infection.List of interaction residues in HLA allele and Gag epitopes were listed in the Table 3 and Figure 2-7.

**Table 3**
***HLA-DRB1*15 allele and Gag Epitope Interaction***

| Epitope | Donor | Acceptor | Bond Type | Bond distance |
|---|---|---|---|---|
| GLNKIVRMYSPTSIL-P1 | DRB1*15:TRP178:NE1 | Gagepitope:SER9:O | Conventional Hydrogen Bond | 1.78141 |
| | DRB1*15:GLU179:N | Gagepitope: MET7:O | | 3.3142 |
| | DRB1*15:TRP178:NE1 | Gagepitope:ASP15:OD | | 3.2961 |
| | Gagepitope:LEU1:N | DRB1*15:HIS177:O | | 3.0989 |
| | Gagepitope:ASN2:ND | DRB1*15:THR90:OG1 | | 2.81841 |
| | Gagepitope:TYR8:OH | DRB1*15:ASP181:OD2 | | 3.37204 |
| | DRB1*15:TRP178:CA | Gagepitope: MET7: O | | 3.42431 |
| | Gagepitope:PRO10:CD | DRB1*15:VAL91: O | | 3.18531 |
| NKIVRMYSPTSILDI-p2 | DRB1*15:LYS105:NZ | Gagepitope:TYR5:OH | Conventional Hydrogen Bond | 2.63133 |
| | DRB1*15:VAL97:N | Gagepitope:GLN15:OXT | | 2.62147 |
| | DRB1*15:TRP178:NE1 | Gagepitope:ILE1:O | | 2.27974 |
| | DRB1*15:THR90:OG1 | Gagepitope:THR8:O | | 2.1305 |
| | Gagepitope:PRO7:CD | DRB1*15:THR145:OG1 | Carbon Hydrogen Bond | 2.4793 |
| | DRB1*15:THR93:CB | Gagepitope:ILE1:O | | 3.74236 |
| | Gagepitope:ARG3:CD | DRB1*15:GLU179:O | | 2.85043 |
| | Gagepitope:GLN15:NE2 | DRB1*15:TYR102 | Pi-Donor Hydrogen Bond | 3.79949 |
| IVRMYSPTSILDIKQ-P3 | Gagepitope:ARG8:NH1 | DRB1*15:ASP110:OD2 | Salt Bridge | 3.7822 |
| | DRB1*15:LYS176:NZ | Gagepitope:GLY2:O | Conventional Hydrogen Bond | 1.84373 |
| | DRB1*15:LYS176:NZ | Gagepitope:VAL7:O | | 3.1078 |
| | DRB1*15:LYS176:NZ | Gagepitope:SER11:OG | | 1.27926 |
| | Gagepitope:LEU1:N | DRB1*15:VAL143:O | | 3.24489 |
| | Gagepitope:LEU1:N | DRB1*15:MET160:SD | | 3.24705 |
| | Gagepitope:GLY2:CA | DRB1*15:SER144:O | Carbon Hydrogen Bond | 3.12132 |
| | Gagepitope:LYS5:CE | DRB1*15:VAL91:O | | 2.05159 |
| LGLNKIVRMYSPTSI-P4 | Gagepitope:ARG8:NH1 | DRB1*15:ASP110:OD2 | Salt Bridge | 3.7822 |
| | DRB1*15:LYS176:NZ | Gagepitope:GLY2:O | Conventional Hydrogen Bond | 1.84373 |
| | DRB1*15:LYS176:NZ | Gagepitope:VAL7:O | | 3.1078 |
| | DRB1*15:LYS176:NZ | Gagepitope:SER11:OG | | 1.27926 |
| | Gagepitope:LEU1:N | DRB1*15:VAL143:O | | 3.24489 |
| | Gagepitope:LEU1:N | DRB1*15:MET160:SD | | 3.24705 |
| | Gagepitope:GLY2:CA | DRB1*15:SER144:O | Carbon Hydrogen Bond | 3.12132 |
| | Gagepitope:LYS5:CE | DRB1*15:VAL91:O | | 2.05159 |
| | Gagepitope:LYS1:N | DRB1*15:GLU88:OE2 | Salt Bridge | 3.01358 |
| | Gagepitope:LYS1:N | DRB1*15:GLU88:OE1 | | 3.83509 |
| | DRB1*15:LYS176:NZ | Gagepitope:ASP13:OD1 | Electrostatic | 4.28938 |
| | DRB1*15:LYS111:NZ | Gagepitope:LYS15:OXT | | 4.8992 |
| | Gagepitope:LYS1:NZ | DRB1*15:GLU88:OE1 | | 5.37386 |
| | Gagepitope:LYS1:NZ | DRB1*15:ASP110:OD2 | | 4.1434 |
| | DRB1*15:TRP178:NE1 | Gagepitope:SER10:OG | Conventional | 2.99836 |

| | | | | |
|---|---|---|---|---|
| KIVRMYSPTSILDIK-P5 | DRB1*15:ASP181:N | Gagepitope:THR9:O | Hydrogen Bond | 2.78994 |
| | Gagepitope:ARG4:NH2 | DRB1*15:HIS177:O | | 2.61581 |
| | Gagepitope:VAL3:CA | DRB1*15:THR145:O | Carbon Hydrogen Bond | 3.2238 |
| | Gagepitope:SER7:CB | DRB1*15:TYR102:OH | | 2.93526 |
| | DRB1*15:MET160:SD | Gagepitope:TYR6 | Pi-Sulfur | 5.36826 |
| | DRB1*15:LEU174 | Gagepitope:ILE2 | Alkyl | 5.13263 |
| | DRB1*15:LYS176 | Gagepitope:ILE2 | | 5.4699 |
| | DRB1*15:LEU92 | Gagepitope:LEU12 | | 5.24373 |
| | DRB1*15:LEU158 | Gagepitope:MET5 | | 4.60754 |
| LNKIVRMYSPTSILD-P6 | DRB1*15:TRP178:NE1 | Gagepitope C:SER9:OG | Conventional Hydrogen Bond | 1.78141 |
| | DRB1*15:GLU179:N | Gagepitope:MET7:O | | 3.31424 |
| | DRB1*15:TRP178:NE1 | Gagepitope:ASP15:OD2 | | 3.2961 |
| | Gagepitope:LEU1:N | DRB1*15:HIS177:O | | 3.09895 |
| | Gagepitope:ASN2:ND2 | DRB1*15:THR90:OG1 | | 2.81841 |
| | Gagepitope:TYR8:OH | DRB1*15:ASP181:OD2 | | 3.37204 |
| | DRB1*15:TRP178:CA | Gagepitope:MET7:O | Carbon Hydrogen Bond | 3.42431 |
| | Gagepitope:PRO10:CD | DRB1*15:VAL91:O | | 3.18531 |

**Figure 2**

***HLA-DRB1*15and epitope P1 interaction analysis and the epitope GLNKIVRMYSPTSIL-P1 binds in the groove of the HLA-DRB1*15 allele.***

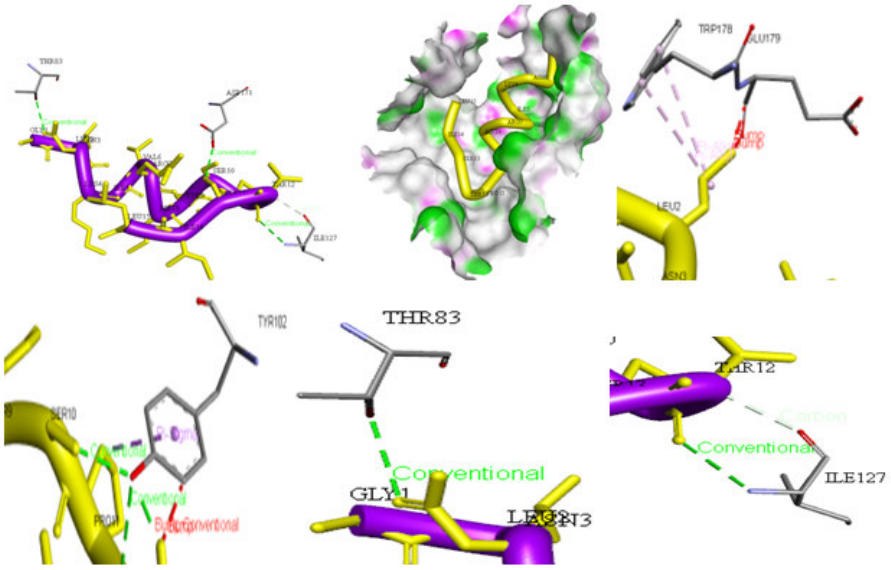


**Figure 3**

***HLA-DRB1*15and epitope P2 interaction analysis and the epitope NKIVRMYSPTSILDI-P2 binds in the groove of the HLA-DRB1*15 allele.***

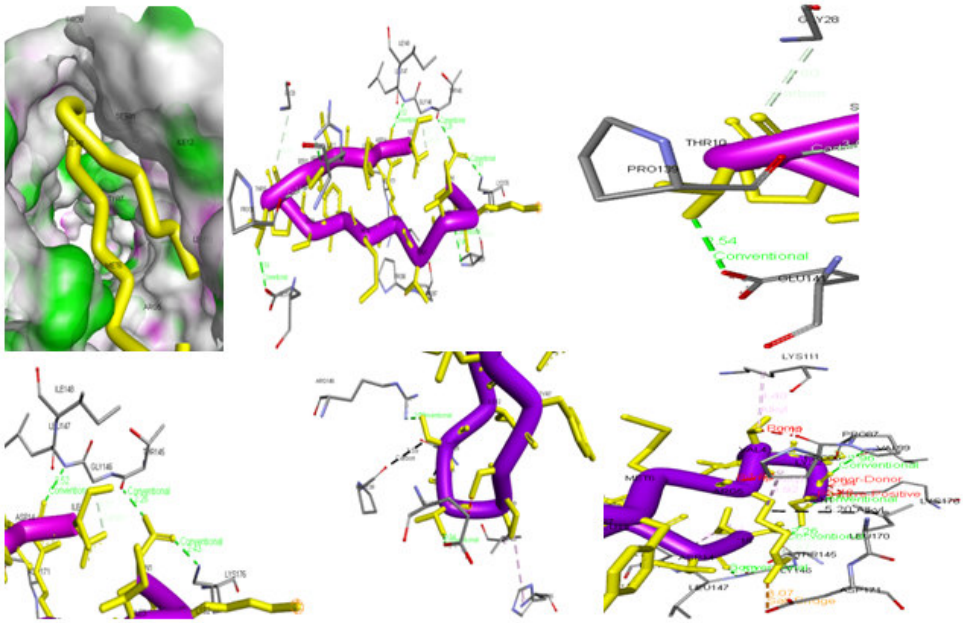



**Figure 4**

***HLA-DRB1* 15and epitope P3 interaction analysis and the epitope IVRMYSPTSILDIKQ binds in the groove of the HLA-DRB1*15 allele.***

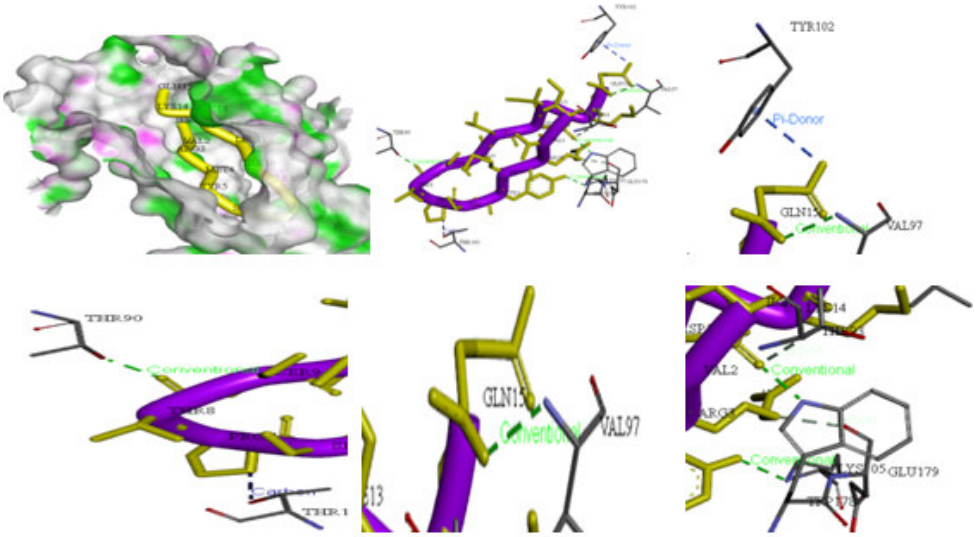


**Figure 5**

***HLA-DRB1*15and epitope P4 interaction analysis and the epitope LGLNKIVRMYSPTSI binds in the groove of the HLA-DRB1*15 allele***

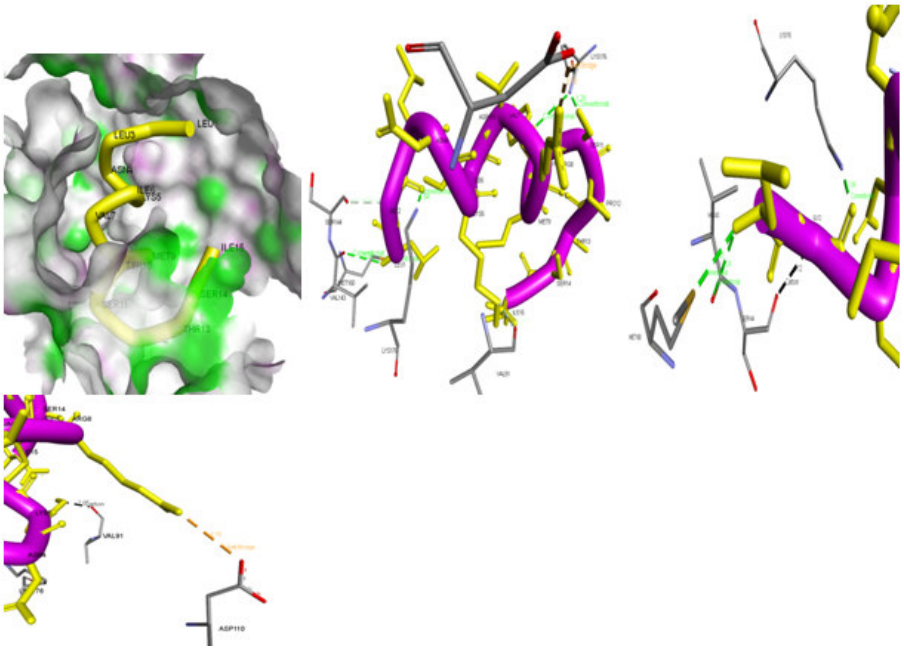


**Figure 6**

***HLA-DRB1*15and epitope P5 interaction analysis and the epitope KIVRMYSPTSILDI binds in the groove of the HLA-DRB1*15 allele***.

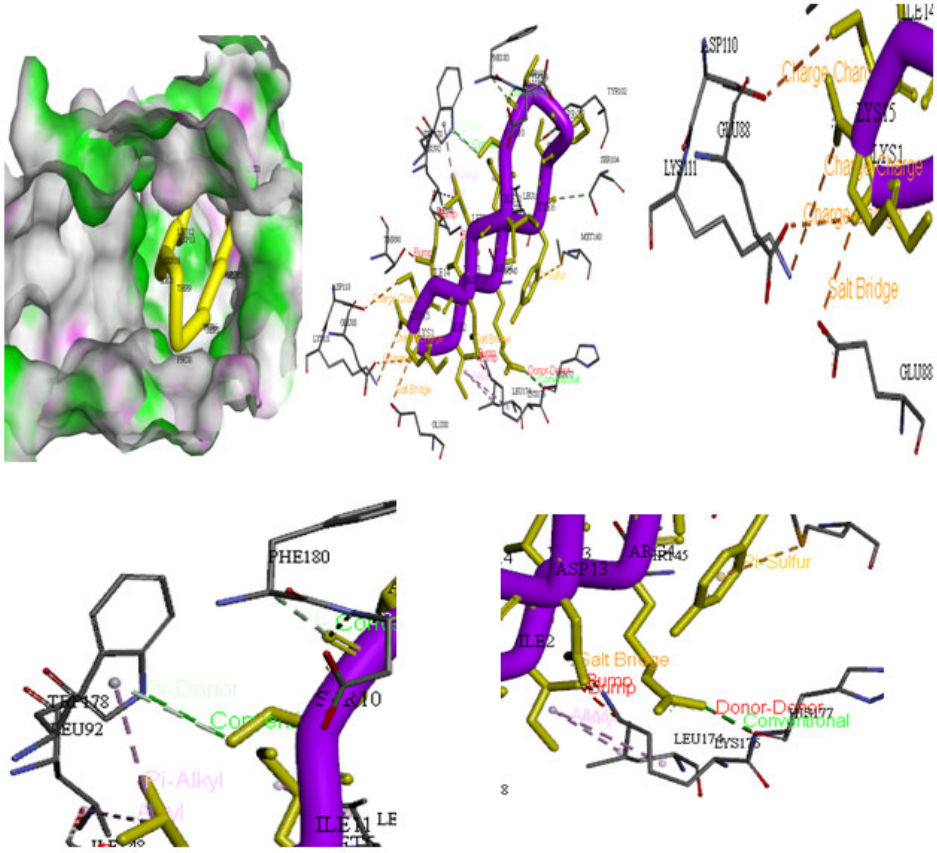
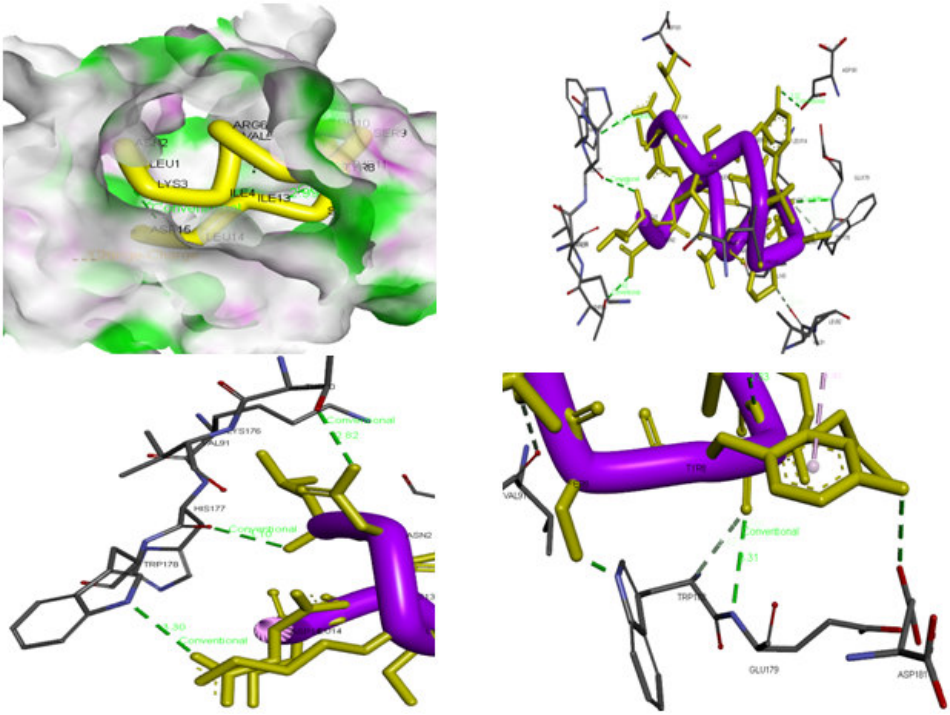


**Figure 7**
***HLA-DRB1*15and epitope P6 interaction analysis and the epitope LNKIVRMYSPTSILD binds in the groove of the HLA-DRB1*15 allele***.



Based on the interaction pattern among the 6 predicted epitopes, it was concluded that P1 ,P2 and P4 were showing the potential to be real epitopes, as their binding orientations in interaction with HIV resistance related HLA-DRB1*15 allele molecules.All 6 epitopes P1-P6 could able occupy the binding grove of the HLA allele

## CONCLUSION

Our studies based on computational approach which combines both epitope prediction and docking techniques provides the structural insight of gag CTL epitopes namely p1(GLNKIVRMYSPTSIL),p2(LGLNKIVRMYSPTSI),p3(LNKIVRMYSPTSILD),p4(NKI VRMY SPTSILDI),p5( IVRMYSPTSILDIKQ), p6(KIVRMYSPTSILDIK) .These CTL Gag based epitopes can be considered as vaccine candidates for HIV infection since they would confer both humoral and cell-mediated immunity.Predicted epitopes resulted with greater affinity for HIV resistance related HLA-DRB1*15 allele and showed population conservancy among Indian population. Immunogenicity of these listed Gag epitopes need to to be tested further in invitro and invivo experiments to construct ideal HIV vaccine for Indian population.

## REFERENCES


1. Moore M. D. and. Hu W. S., HIV-1 RNA dimerization: it takes two to tango. AIDS Reviews, 11, (2): 91–102,( 2009)
2. United Nations Programme on HIV/AIDS. “Global Report fact sheet: the global AIDS epidemic”,2013. http://www.unaids.org/en/resources/campaigns/globalreport2013/factsheet/
3. “NACO annual report/”,2013-14. http://naco.gov.in/NACO/Quick_ Links/Publication/Annual_

Report/NACO_Annual_Report/Annual_Report_2013-14/
4. Kumarasamy N, Patel A, Pujari S., Antiretroviral therapy in Indian setting: When & that to start with, when & what to switch to?. Indian J Med Res,134:787–800(2011)
5. Cohen J., Shots in the dark: the wayward search for an AIDS vaccine. New York: W.W. Norton & Company,(2001)
6. Miedema F., A brief history of HIV vaccine research: stepping back to the drawing board? AIDS. 22(14),1699–703(2008)
7. Grady C., The Search for an AIDS vaccine: ethical issues in the development and testing of a preventive HIV vaccine. 1. Bloomington and Indianapolis (IN): Indiana University Press; (1995).
8. Spearman P., HIV vaccine development: lessons from the past and promise for the future. Curr HIV Res.;1(1):101–20,( 2003)
9. Munier CM, Anderson CR, Kelleher AD. HIV vaccines: progress to date. Drugs.;71(4):387–414,( 2011)
10. HIV Vaccines and Microbicides Resource Tracking Working Group.;"Investing to end the AIDS epidemic: a new era for HIV prevention research & development. "2013Apr16"http://www.hivresourcetracking.org/sites/default/files/July%202012%20Investing%20to%20End%20the%20AIDS%20Epidemic%20A%20New%20Era%20for%20HIV%20Prevention%20Research%20&%20Development.pdf
11. Costin. J. M., "Cytopathic mechanisms of HIV-1," Virology Journal, vol. 4, article 100,( 2007)
12. Barouch DH., Challenges in the development of an HIV-1 vaccine. Nature 455:613–619, (2008)
13. Walker BD, Ahmed R, Plotkin S .,Moving ahead an HIV vaccine: use both arms to beat HIV. Nat Med 17:1194–1195, (2011)
14. Thomson M. M, Pérez-Álvarez L, and Nájera R., "Molecular epidemiology of HIV-1 genetic forms and its significance for vaccine development and therapy," The Lancet Infectious Diseases, v 2, (8), 461–471,( 2002)
15. Hemelaar J, Gouws E, Ghys PD, Osmanov S, WHO-UNAIDS network for HIV isolation and characterisation (2011) Global trends in molecular epidemiology of HIV-1 during 2000–2007. AIDS 25:679–689
16. Brusic V and. August J. T, "The changing field of vaccine development in the genomics era," *Pharmacogenomics*, 5,( 6) 597–600,( 2004).
17. Ovsyannikova I. G.,. Jacobson R. M, and. Poland G. A, "Variation in vaccine response in normal populations," *Pharmacogenomics*, 5(4)417–427, (2004)
18. MacDonald KS, Fowke KR, Kimani J, Dunand VA, Nagelkerke NJ,Ball TB, Oyugi J, Njagi E, Gaur LK, Brunham RC, Wade J,Luscher MA, Krausa P, Rowland-Jones S, Ngugi E, Bwayo JJ, Plummer FA .,Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. J Infect Dis 181:1581–1589, (2000)
19. Singh P, Kaur G, Sharma G, Mehra NK .Immunogenetic basis of HIV-1 infection, transmission and disease progression. Vaccine 26:2966–2980, (2008)
20. Selvaraj P, Swaminathan S, Alagarasu K, Raghavan S, Narendran G, Narayanan P .,Association of human leukocyte antigen-A11 with resistance and B40 and DR2 with susceptibility to HIV-1 infection in south India. J Acquir Immune Defic Syndr 43:497–499 (2006)
21. Kaslow RA, Dorak T, Tang JJ., Influence of host genetic variation on susceptibility to HIV type 1 infection. J Infect Dis 191:S68–S77, (2005)
22. McNeil AJ, Yap PL, Gore SM, Brettle RP, McColl M, Wyld R, Davidson S, Weightman R, Richardson AM, Robertson JR., Association of HLA types A1-B8-DR3 and B27 with rapid and slow progression of HIV disease. QJM 89:177–18, (1996)
23. Neumann-Haefelin C., Protective role of HLA-B27 in HIV and hepatitis C virus infection, Dtsch Med Wochenschr 136:320–324, (2011)
24. nternational HIV Controllers Study, Pereyra F, Jia X, McLaren PJ,Telenti A, de Bakker PI, Walker BD, Ripke S, Brumme CJ, Pulit SL, Carrington M et al .,The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science 330:1551–1557, (2010)
25. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, Hallahan CW, Selig SM, Schwartz D, Sullivan J, Connors M .,HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term

nonprogressors. Proc Natl Acad Sci U S A 97:2709–2714, (2000)
26. Tomiyama H, Sakaguchi T, Miwa K, Oka S, Iwamoto A, Kaneko Y,Takiguchi M., Identification of multiple HIV-1 CTL epitopes presented by HLA-B*5101 molecules. Hum Immunol ,60:177–186, (1999)
27. Zhang Y, Peng Y, Yan H, Xu K, Saito M, Wu H, Chen X, Ranasinghe S, Kuse N, Powell T, Zhao Y, Li W, Zhang X, Feng X, Li N, Leligdowicz A, Xu X, John M, Takiguchi M, McMichael A, Rowland-Jones S, Dong T., Multilayered defense in HLAB51-associated HIV viral control. J Immunol 187:684–691, (2011)
28. Ranasinghe S, Cutler S, Davis I, Lu R, Soghoian DZ, Qi Y, Sidney J, Kranias G, Flanders MD, Lindqvist M, Kuhl B, Alter G, Deeks SG, Walker BD, Gao X, Sette A, Carrington M, Streeck H., Association of HLA-DRB1-restricted $CD4^+$ T cell responses with HIV immune control. Nat Med;19(7):930-3,( 2013)
29. Christy and Anand: Analysis of the HIV resistance related HLA dRB1 restricted CD4+ T cell epitopes among Indian HIV patients. BMC Infectious Diseases, 14(Suppl 3):O4, (2014)
30. Kuiken C, Korber B, and Shafer R W, "HIV sequence databases," *AIDS Reviews*, 5( 1)52–61, (2003)
31. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R .,Analysis Tool Web Services from the EMBL-EBI.Nucleic acids research 2013 Jul;41:W597-600, *(2013)*
32. Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, Lopez R *.,A new bioinformatics analysis tools framework at EMBL-EBI* Nucleic acids research 2010 Jul, 38 Suppl: W695-9 , *(2010)*
33. Vita R, Zarebski L, Greenbaum J. A. et al., "The immune epitope database 2.0," *Nucleic Acids Research*, 38, D854– D862, (2010)
34. Wang P, Sidney J, Kim Y et al., "Peptide binding predictions for HLA DR, DP and DQ molecules," *BMC Bioinformatics*, 11, 568, (2010)
35. Nielsen M, Lund O., NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. *BMC Bioinformatics.* 10:296,( 2009)
36. Nielsen M, Lundegaard C, Lund O., Prediction of MHC class II binding affinity using SMM-align, a novel stabilization matrix alignment method. *BMC Bioinformatics.* 8:238(2007)
37. Sturniolo T, Bono E, Ding J, Raddrizzani L, Tuereci O, Sahin U, Braxenthaler M, Gallazzi F, Protti MP, Sinigaglia F, Hammer J.. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. *Nat Biotechnol.* 17(6):555-561,( 1999)
38. Karosiene E1, Rasmussen M, Blicher T, Lund O, Buus S, Nielsen M., NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. *Immunogenetics* 65(10)711,( 2013)
39. Bui HH,Sidney J, Dinh K, Southwood S, Newman MJ, Sette A., Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC Bioinformatics. Mar 17; 7:153,( 2006)
40. Gonzalez-Galarza F. F, Christmas S, Middleton D, and Jones A.R., "Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations," *Nucleic Acids Research*, 39( 1) D913–D919,( 2011)
41. P. Thévenet, Y. Shen, J. Maupetit, F. Guyon, P. Derreumaux, and P. Tufféry, "PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides," Nucleic Acids Research, 40(1) W288–W293,( 2012)
42. Berman H.M, Westbrook J, Feng Z, Gilliland G, Bhat T.N, Weissig H, Shindyalov I.N, Bourne P.E.,The Protein Data Bank.Nucleic Acids Research, 28 ,235-242 (2000)
43. Tovchigrechko A , Vakser IA. [2006] GRAMM-X public web server for protein-protein docking. Nucleic Acids Res,34:W310–314.
44. Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, San Diego: Accelrys Software Inc., 2013.