

# Screening of Fascaplysin for its Efficient Abatement of Hepatocellular Carcinoma and Promiscuous Epitope Mapping of Selected Proteins with Docking Perspectives.

Sethuraman Naveenkumar<sup>1</sup>, Neelamegam Rameshkumar<sup>2</sup>, Nagarajan Kayalvizhi<sup>3</sup>, Natesan Manoharan<sup>1\*</sup>

## \*Corresponding author:

Natesan Manoharan

<sup>1</sup> Department of Marine Science, Bharathidasan University, Tiruchirappalli – 620024, Tamilnadu, India.

<sup>2</sup> Department of Animal Science, Bharathidasan University, Tiruchirappalli – 620024, Tamilnadu, India.

<sup>3</sup> Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli – 620024, Tamilnadu, India.

## Abstract

Hepatocellular carcinoma (HCC) is regarded as one among the deadliest cancers in the world. In particular, aflatoxin induced hepatocellular carcinoma is alarmingly on rise due to food contamination. However, several drugs and synthetic compound are reported to inhibit HCC in humans. Fascaplysin is a marine sponge derived compound has been increasingly considered and proved as a potential inhibitor of CDK2/CDK4 dependent kinase. In the present study, five proteins that are involved in activation of aflatoxin B1 induced hepatocellular carcinogenesis were selected and analyzed for its immunological profiling. Crystal structure of Human Glutathione S-Transferase (GST) A1, Prostaglandin H2 Synthase (PHS), Human Cytochrome p450 3A4, Human Microsomal P450 1A2 and p53 were assessed for their immunogenicity patterns. On the other hand, HLA B27 allele which plays a crucial role in cancer was chosen for T cell and B cell epitope mapping. In this analysis, it shows that SYFPEITHI immunogenic peptides were conserved in all the proteins envisaging the need for a common anti-cancer ligand. However, immunogenic assessment of epitopes and interacting residues revealed that fascaplysin interacts at multiple positions in binding amino acid residues of the selected proteins. This study is purely based on the immunoinformatics approach for the screening of specific compound which could suppress the hepatocellular carcinoma. Therefore, based on the results it's clear that fascaplysin is a potential inhibitor and effectively binds to immunogenic peptides and act as a candidate against aflatoxin induced hepatocellular carcinoma based on the Insilco analysis.

**Keywords:** Aflatoxin, Hepatocellular carcinoma, Fascaplysin, Immunoinformatics, Epitope mapping.

## Introduction

Cancer is one among the highly mortal disease that causes death worldwide and was estimated to escalate death toll to 12 million in 2030 [1]. Aflatoxins are toxic secondary metabolites present in contaminated foods affecting human health and livestock in tropical regions [2, 3]. Aflatoxin B1 (AFB1) produced by *Aspergillus flavus* is toxic to liver and responsible for diseases like toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma [4, 5]. Hepatocellular Carcinoma (HCC) still needs a lot of novel abatement procedures and technologies to overcome morbidity and mortality. Selective inhibition of cancer cells with reduced toxicity to normal cells requires lot of advances. Some interesting discoveries viz, anticancer drug encapsulation by delivery system [6] monoclonal antibody mediated targeting [7, 8] and cell specific peptide ligands against cancerous cells were also reported earlier [9, 10]. The activation of aflatoxin has been demonstrated as an important step for cytotoxic and genotoxic effects. The metabolism

of AFB1 is activated by cytochrome p450 (CYP450s) enzymes and its leads to exo-8, 9 epoxide formations. Especially the cytochrome P450 enzymes are mainly involved in the oxidative biotransformation of AFB1. Among, several human CYP450 enzymes CYP1A2 and CYP3A4 are primarily responsible for the activation of AFB1 to epoxide formations [11–13]. Immune therapy against cancer includes adoptive transfer of *ex vivo* expanded antigen specific T cells and *in vivo* vaccination [14-17]. Antigen presentation is the critical step in immunoinformatics studies and provides a significant breakthrough in T cell based cancer therapies [18]. The era of immunoinformatics has witnessed significant development and applications in the clinical field [19]. *In silico* predictions of T cell epitopes have been successfully applied to design new vaccines and autoimmunity studies [20-22]. Nevertheless, Major Histocompatibility (MHC) restriction to epitope based vaccines design emphasizes the need for immunogenic peptide prediction.



Fascaplysin derived from the marine sponge *Fascaplynopsis* sp has selectively inhibits CDK4 than CDK2. Molecular insights based on thermodynamic integration revealed that Histidine 95 of CDK4/CDK2 may play a crucial role in selectivity of inhibition since fascaplysin is positively charged. Therefore, maintaining a positively charged functional group is important in CDK4 inhibitors design [23]. In the present study, five proteins involved in HCC were selected namely cytochrome p450 3A4, human microsomal p450 1A2 has been mainly present in the wide range of malignant tumors especially in HCC [24]. Prostaglandin H2 synthase is a crucial protein for causing human pancreatic adeno carcinoma cells [25]. P53 protein is a major oncogenic protein, strongly suggesting that downstream effector of large T antigen pathway and high level of p53 gene expression is found in all cancer cells [26]. GST has been associated with multidrug resistant tumor cells and over expression of GST's can increase susceptibility to carcinogenesis and inflammatory diseases [27]. The study aimed on the fact that epitope based vaccine approach exploits immune

system functioning and immunoinformatics [28, 29]. The main objective of the present study is to development of computational approach in developing epitope based vaccinations in arresting a HCC. In view of the above, the screening of fascaplysin as a potent anti-therapeutic agent against Hepatocellular carcinoma and immunogenic profiling of SYFPEITHI prediction of conserved immunogenic peptides through T cell and B cell epitope mapping was carried out. The immunogenic peptides are then counter checked and validated through docking perspectives to evaluate the binding efficacies and ligand affinities with fascaplysin (Figure1). Nevertheless, aflatoxin induced HCC is least studied on the conservancy scale. To the best of our knowledge, HCC immunoinformatics precisely has not been studied earlier and this report would be first of its kind in addressing this issue. The immunoinformatics approach employed in this study will be a valuable tool for the generation of hypothesis for the selection of biological and immunological targets for experimental analysis. This would have high impact in anticancer drug vaccination.

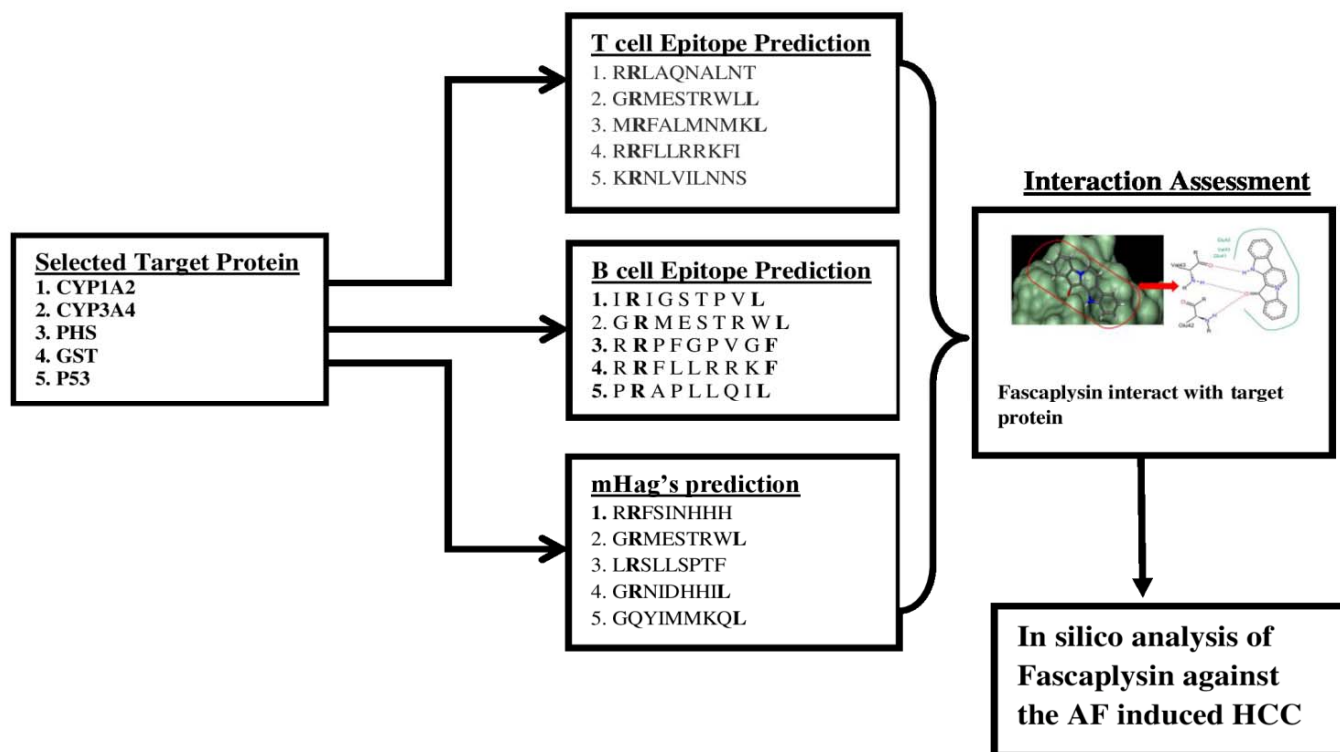


Figure 1: Schematic Representation of over all work flow

## Materials and Methods

### Protein selection

Protein structure of significant cancer related proteins were retrieved from PDB database (<http://www.rcsb.org/pdb/>). The

proteins selected for the analysis were 1PKZ - Crystal structure of human Glutathione S Transferase (GST) A1, 1PRH – Prostaglandin H2 Synthase, 1W0E - Crystal structure of human Cytochrome p450 3A4, 2HI4 - Crystal Structure of Human Microsomal P450 1A2 and 2Z5T- p53. These proteins were



significant in aflatoxin induced hepatocellular carcinogenesis. Secondary structure prediction of membrane of these five proteins is determined by SOSUI server (<http://bp.nuap.nagoya-u.ac.jp/sosui/>).

### T cell epitope prediction

Binding strength of T cell epitope to major histocompatibility complex (MHC or HLA) is a significant determinant of immunogenicity. Moreover, it allows displaying of higher binding affinity T cell epitopes for recognition with T cell receptor [30] For identification of immunogenic T cell epitopes, IEDB was used for assessing MHC class I and class II epitopes ([http://tools.immuneepitope.org/analyze/html/mhc\\_binding.html](http://tools.immuneepitope.org/analyze/html/mhc_binding.html)). IEDB involves utilization of ANN algorithm utilizing Stabilized Matrix Method (SMM). Non overlapping epitopes were selected as possible and potent epitopes [31].

### B cell epitope prediction

B cell epitope prediction by BCPRED (<http://ailab.cs.iastate.edu/bcpreds/predict.html>) was performed on the basis of *ex vivo* analysis. Epitopia server (<http://epitopia.tau.ac.il/>) incorporates a machine learning based algorithm for predicting immunogenic B cell epitopes. Conserved B cell epitopes were selected as conformational epitopes.

### Accessibility prediction

Accessibility involves disparity between solvent accessible surface area and the area for immunogenicity on the protein surfaces. The solvent accessible surface area was analyzed employing Kyte and Doolittle method GETAREA (<http://curie.utmb.edu/getarea.html>).

### Prediction of minor histocompatibility antigens (or mHag's prediction)

Minor histocompatibility antigen predictions are significant in analyzing mutational parameters and variations in single nucleotide polymorphism that contribute to carcinogenesis. mHag's are peptides presented by MHC that is responsible for alloreactive immune response. Sipep (<http://igrid-ext.cryst.bbk.ac.uk/snp/pro.php>) which utilize coding non synonymous SNPs mutation with data form dbMHC and Hap Map projects was used in the present study. The methodology described here employs SYFPEITHI mode of HLA binding [18].

### Preparation of ligands

The ligand chosen was retrieved from the PUBCHEM data base (<http://pubchem.ncbi.nlm.nih.gov/>) and the 3D structures were generated using arguslab. RMSD based energy minimization was performed in *vacuo* to give a first optimization of the rough structure using VEGA ZZ [32]. Hydrogen was initially added to receptor molecule, AMBER and gasteiger charges were added to fix unusual bonds in the 3D structure which utilizes CHARMM force field parameters.

### Interaction Assessment

Patch dock was used for interaction assessment with a default value RMSD 4.0. Patch dock is a molecular docking algorithm based on shape complementarity principle. The highest scores were taken as significant interaction with less atomic contact energy (ACE). Hydrogen bond interactions were assessed by MOLEGRO (<http://www.molegro.com/>). The active site binding to faspaplysin was assessed by Leadit functions of FLEXX docking with flexible residues (<http://www.biosolveit.de/FlexX/>).

### Result and Discussion

#### T cell epitope prediction

In this study, epitope mapping is restricted to HLA B27 as the association might share a pathogenic pathway in aflatoxin induced HCC [33]. The atomic SASA covered by each cleft was calculated by utilizing radius of water probe 1.4 Å and the area/energy per residue was calculated. When the protein surface was exposed for major solvent accessible surface area it reflects in the hindering of immunogenic response in *in vivo*. The Area/ Energy per residue were calculated which indicates the higher energy for prostaglandin H2 synthase (40911.93) than other proteins. The result confirmed the fact that accessible surface area increases the immunogenicity. The SASA result for glutathione S- transferase shows it is an apolar rich residue that made us unable to calculate area/energy per residue. Our results clearly indicated that the higher solvent accessible surface area leads to higher level of immunogenicity and SASA results tabulated in (Table 1a) also confirm the same. Immunogenic peptides for identification of vaccines candidate by employing traditional molecular immunology techniques were expensive and time consuming [34]. However, bacterial recombinant attenuated vaccines are potent and safe in developing immune strategy.

**Table 1a. Solvent accessible surface area (SASA)**

S.No	Proteins name	Area/Energy Per Residue
1	Cytochrome P450 1A2	20928.46
2	glutathione S transferase (GST) A1	-nan*
3	Cytochrome P450 3A4	19450.28
4	prostaglandinH2synthase	40911.93
5	P53	16311.47

-nan\* denotes apolar rich residues that renders GST with no solvent accessibility

On the other hand, emerging immunoinformatics has led to increased discovery of cancer immune therapies [35]. Based on the analysis among five proteins, cytochrome p450 1A2 is a transmembrane protein and the remaining were soluble proteins. The flexibility, hydrophilicity, polarity and surface properties at a threshold value of 2.38 were employed for accurate prediction. The analysis of T cell epitope mapping showed highly conserved region



of R (Arginine) in the second position of predicted MHC I binding region. Based on IC50 values, prostaglandin H2 synthase and glutathione S-transferase A1 showed best binding affinity at 0.10 nM than the other proteins (Table 1b). The analysis of the varied nature of binding characteristics the chosen proteins with MHC II molecules clearly depicts in Table 1c. Binding affinities of T cell

epitopes predicted may be due to the phenomenon of enthalpy-entropy compensation [36]. T cell epitope mapping provided a rough map of MHC I and MHC II binding peptides. The peptides identified were not conserved and hence B cell epitope prediction was sought.

**Table 1b. MHC –I binding Prediction based on artificial neural network and simplified matrix methods.**

S.No	Proteins Name	Allele	Start	End	Peptide Length	Sequence	IC 50 value in nM
1	Cytochrome P450 1A2	HLA-B*27:05	113	122	10	RRLAQNALNT	0.20
2	Glutathione S transferase (GST) A1	HLA-B*27:05	14	23	10	GRMESTRWLL	0.10
3	Cytochrome P450 3A4	HLA-B*27:05	423	432	10	MRFALMNMKL	0.15
4	prostaglandinH2synthase	HLA-B*27:05	147	156	10	RRFLLRRKFI	0.10
5	P53	HLA-B*27:05	100	109	10	KRNLVILNNS	3.00

**Table 1c. MHC –II binding Prediction based on NetMHC pan II.**

S.No	Proteins Name	Allele	Start	End	Sequence	IC 50 value in nM
1	Cytochrome P450 1A2	HLA-B*27:05	214	228	FFPILRYLPNPALQR	0.27
2	Glutathione S-Transferase (GST) A1	HLA-B*27:05	177	191	SFPLLKALKTRISNL	0.53
3	Cytochrome P450 3A4	HLA-B*27:05	367	381	PKGVVVMIPSYALHR	0.31
4	Prostaglandin H2 Synthase	HLA-B*27:05	256	270	GQEVFGLLPGLMLYA	0.09
5	P53	HLA-B*27:05	100	114	KRNLVILNNSDAAKN	0.92

### B cell epitope prediction

The B cell epitopes showed varied and diversified presence of amino acid indicate its cell type. The Immunogenic residues were recognized employing SYFPEITHI predictions. The epitopes were found within the range of 23 – 31 epitopes with highest corresponding to prostaglandin H2 synthase with 31 epitopes and

least for p53. Low number of epitopes for p53 is due to the fact that it is a tumor suppressor protein. SYFPEITHI predictions reveal that R (Arginine) in the second position and F (Phenylalanine) and L (Leucine) are highly conserved among all the proteins involved in HCC was reported in Table 2a.

**Table 2a. SYFPEITHI Predictions of Immunogenicity**

S.No	Proteins Name	Position	Epitope	No. of epitopes
1	Cytochrome P450 1A2	54	I R I G S T P V L	28
2	Glutathione S transferase (GST) A1	14	G R M E S T R W L	25
3	Cytochrome P450 3A4	83	R R P F G P V G F	27
4	Prostaglandin H2 synthase	147	R R F L L R R K F	31
5	P53	26	P R A P L L Q I L	23



Interestingly, in both T cell and B cell prediction the R (Arginine) in the second position is highly conserved it might be significant for immunogenic nature. The role of R (arginine) and L (Leucine) present in the binding pockets are specially indicated for ligand binding. The role of two residues are counter balanced in the binding packets depending on the chemical structure of ligand in which it gives flexibility to the binding pocket and allow binding to ligand [39]. In the present study, faspaplysin is a ligand and also R (arginine) and L (Leucine) are present in the binding pocket of the target protein, therefore it might play a crucial role in the ligand binding. Furthermore, profiling of predicted epitopes that are responsive to proteosomal cleavage was performed by minor histocompatibility antigen prediction. Proteosomal cleavage assessment relies on the fact that a complex pathway was involved in proteolysis [37, 38].

### Prediction of minor histocompatibility antigens (or) mHag's prediction

Table 2b. Prediction of minor histocompatibility antigens (or) mHag's prediction

S.No	Proteins Name	Position	Peptide	Proteosomal Cleavage	CombiPred	NHLAPred
1	Cytochrome P450 1A2	527	RRFSINHHH	0	5.8	-2.65
2	Glutathione S transferase (GST) A1	43	GRMESTRWL	6.3	4.08	-5.35
3	Cytochrome P450 3A4	138	LRSLLSPTF	6.59	4.76	-4.28
4	Prostaglandin H2 synthase	444	GRNIDHHIL	6.24	5.06	-3.82
5	P53	83	GQYIMMKQL	6.44	2.99	-7.06

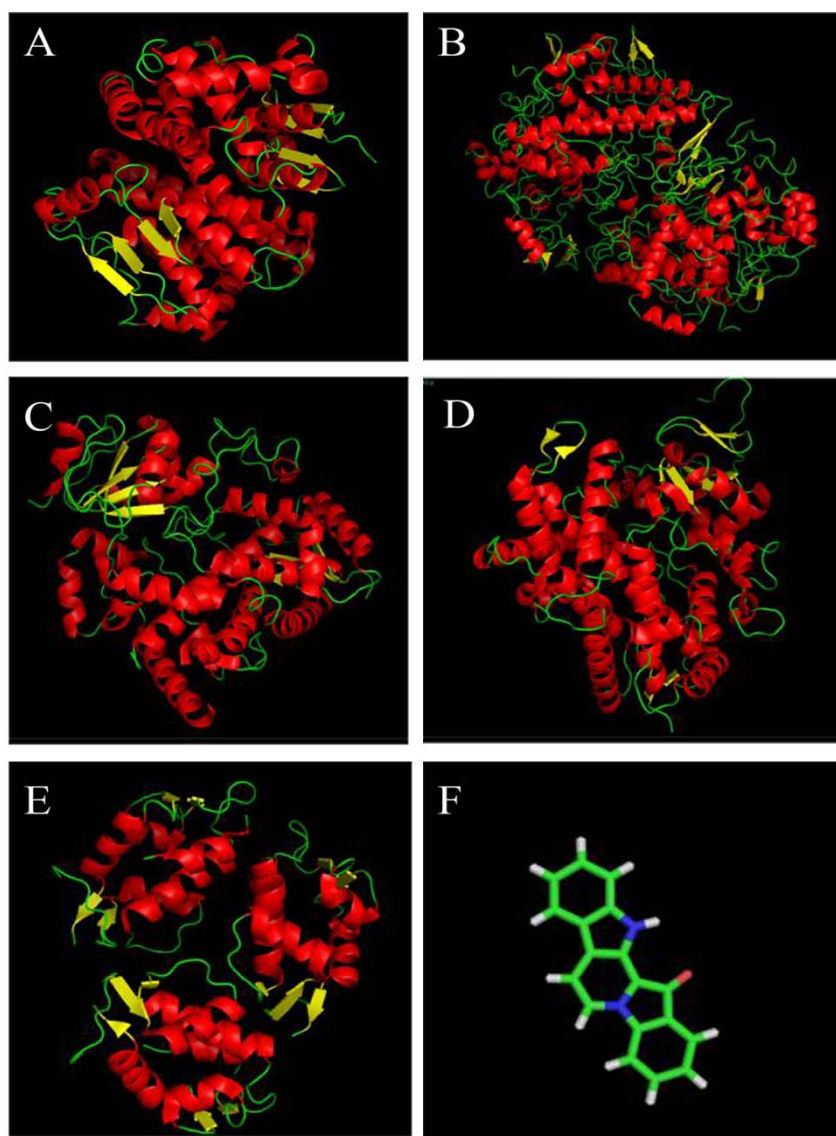
### Interaction Assessment

The interaction study was performed by rigid protein target and flexible ligand. The selected target proteins were predicted for its immunogenic regions. The immunogenic residues found in the epitopes predicted were inspected manually and found p53 had no efficient binding assessed by the least score of interaction and all other proteins were found to possess the immunogenic residues in the binding site of the docked complex. The lower binding scores and increased ambiguity renders the docked complex as highly stable. Figure 2 shows the 3D models of selected proteins and ligand. Based on the docking study cytochrome p450 1A2 had the highest binding and ambiguity score for the faspaplysin was tabulated in table 3. Figure 3, indicates the interaction studies in the displacement of water and its role in the stable interaction. Cytochrome p450 3A4 had water displaced at Cys 422 and Gly 444 positions interestingly Phe, Gly, Val and Ile were dominated in the binding region. GST had Ser at 18th position in the water displacement site majority of interacting residues belongs to Thr, Ser, Gly, Arg, Glu and Leu. On the other hand, cytochrome p450 1A2 displaced the water molecule and shows interaction with Leu

Predicted antigenic peptides that can bind to minor histocompatibility antigens were conserved with R (arginine) in the second position and L (Leucine) in the ninth position. However, proteosomal cleavage was absent in cytochrome p450 1A2 an ample proteosomal cleavage depicts the mHag's based on SNP's in Table 2b. CombiPred and NHLAPred defines the proteosomal cleavage and it holds good for all the proteins except for cytochrome p450 1A2. This might be correlated with peptide position as 527th position is so far from the start site. In general, T-cell epitope are protein fragments present in the cell surface which trigger immune system to detect and respond immediately, however in the present study the faspaplysin prefer both T and B cell epitope binding regions of cytochrome p450 1A2 protein. Further, the chosen proteins were subjected to docking to assess the inhibitory nature of the drug.

450, Gln 411 and also interact with other amino acids present in the active site (Gly, Thr and Leu). Similarly, prostaglandin H2 synthase had His 207 and Gln 289 in the displaced portion with Phe and Lys in the realm of interaction. All the proteins chosen had a positive interaction, except for p53 which is a tumor suppressor protein. While analyzing the protein and ligand interaction the conserved amino acids R (Arginine) found at the interacting site based on the T cell and B cell interaction and correlation with interactions studies it clears that Arginine and Lysine might play a key role in interaction of protein and faspaplysin to inhibit HCC. Sorafenib, an inhibitor targeting vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and Raf signaling pathways is the only available drug prolonging survival of HCC patients [40 - 41]. Manual inspection of the immunogenic peptides profiles among the interacting residues. Docking perspectives of faspaplysin reveals that it inhibits HCC, as the immunogenic peptides are majorly involved in binding pockets of the docked complexes.



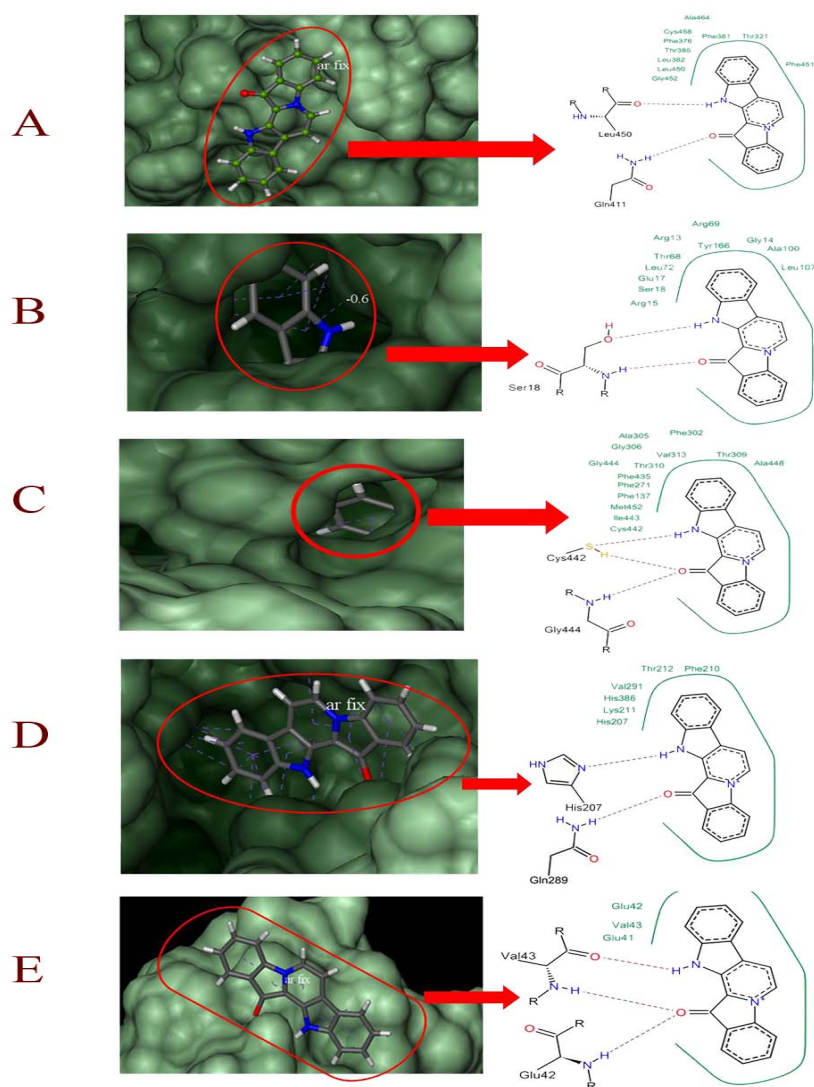


**Figure 2:** Proteins and ligand used in the analysis A) Crystal Structure of Human Microsomal P450 1A2, B) Crystal structure of human Glutathione S-Transferase (GST) A1, C) Crystal structure of human Cytochrome p450 3A4, D) Prostaglandin H<sub>2</sub> Synthase , E) p53 and F) Fascaplysin.

**Table 3. Flex score and Binding energy of Docked complexes**

S.No	Proteins Name	Score	Ambiguity
1	Cytochrome P450 1A2	-29.0422	-5.8292
2	Glutathione S transferase (GST) A1	-22.2598	-5.7603
3	Cytochrome P450 3A4	-21.3188	-4.5851
4	Prostaglandin H2 synthase	-20.3068	-6.5846
5	P53	-16.5255	-4.0533





**Figure 3:** Docked complexes of the five proteins with fascaplysin showing potent interactions with displaced water residues.

## Conclusion

Immunoinformatics serves as a valuable tool to screen and select antigenic peptides as a potential T cell and B cell epitope for finding affinity with these selected target proteins. Fascaplysin is an efficient abatement tool to combat HCC. Immunogenic profiling revealed that epitopes specific for eliciting an immune response. In this study the cytochrome p450 1A2 had higher binding to fascaplysin with this interaction result it might be the right pathway to inhibit the AFB1 induced HCC. Lessened toxicity of fascaplysin was assessed by the earlier report suggested that up to 10 mg/kg, of fascaplysin was not toxic to mice [42]. Our results were also consistent with the above report suggesting that fascaplysin was less toxic. The approach employed in the present study will be a

valuable tool for the generation of scientific hypothesis in the selection of biological and immunological targets for experimental testing. This analysis could be extended for all cancers and design of novel DNA vaccines based on immunogenic profiling.

## Conflict of Interest statement

No conflict of interest among the authors in this manuscript.

## Acknowledgement

S. Naveenkumar acknowledges University Grants Commission, India for grant in the form of fellowship.



## References

- [1]. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. *CA Cancer J Clin.* 2010; 5: 277–300.
- [2]. Kobayashi T, Abe K, Asai K, Gomi K, Juvvadi PR. Genomics of *Aspergillus oryzae*. *Biosci Biotechnol. Biochem* 2007; 71: 646–670.
- [3]. Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* 2005; 438: 1151–1156.
- [4]. Reddy KRN, Reddy CS, Muralidharan K. Characterization of aflatoxin B1 produced by *Aspergillus flavus* isolated from discolored rice grains. *J Mycol Plant Pathol* 2005; 35: 470–474.
- [5]. Speijers GJA, Speijers MHM. Combined toxic effects of mycotoxins. *Toxicol Lett* 2004; 153: 91–98.
- [6]. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science.* 2004; 303: 1818–1822.
- [7]. Allen TM, Mumbengegwi DR, Charrois GJ. Anti-CD19-targeted liposomal doxorubicin improves the therapeutic efficacy in murine B-cell lymphoma and ameliorates the toxicity of liposomes with varying drug release rates. *Clin Cancer Res.* 2005; 11: 3567–3573.
- [8]. *Mac Diarmid* JA, Mugridge NB, Weiss JC, Phillips L, Burn AL. et al., Bacterially derived 400 nm particles for encapsulation and cancer cell targeting of chemotherapeutics. *Cancer Cell.* 2007;11: 431-445.
- [9]. Lee TY, Wu HC, Tseng YL, Lin CT. A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery. *Cancer Res.* 2004; 64: 8002–8008.
- [10]. Xiong XB, Huang Y, Lu WL, et al., Enhanced intracellular delivery and improved antitumor efficacy of doxorubicin by sterically stabilized liposomes modified with a synthetic RGD mimetic. *J Control Release.* 2005; 107: 262–275.
- [11]. Aoyama T, Yamano S, Guzelian PS, Gelboin HV. Five of 12 forms of vaccinia virus-expressed human hepatic cytochrome P450 metabolically activates aflatoxin B1. *Proc. Natl Acad Sci.* 1990; 87: 4790–4793.
- [12]. Crespi CL, Penman BW, Steimel DT, Gelboin HV, Gonzalez FJ. The development of human cell line stably expressing human CYP3A4; role in the metabolic activation of aflatoxin B1 and comparison to CYP1A2 and CYP2A3. *Carcinogenesis.* 1991; 12: 355–359.
- [13]. Shimada T, Guengerich FP. Evidence for cytochrome P-450NF, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proc. Natl Acad Sci.* 1989; 86: 462–465.
- [14]. Yee C. Adoptive T cell therapy: addressing challenges in cancer immunotherapy. *J Transl Med.* 2005; 3: 17.
- [15]. Knutson KL, Wagner W, Disis ML. Adoptive T cell therapy of solid cancers. *Cancer Immunol Immunother.* 2006; 55: 96–103.
- [16]. Ribas A, Timmerman JM, Butterfield LH, Economou JS. Determinant spreading and tumor responses after peptide-based cancer immunotherapy. *Trends Immunol.* 2003; 24: 58-61.
- [17]. Maus MV, Thomas AK, Leonard DG, et al., *Ex vivo* expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. *Nat Biotechnol.* 2002; 20: 143–148.
- [18]. De Luca DS, Blasczyk R. The immune-informatics of cancer immunotherapy. *Tissue Antigens.* 2007; 70: 265–271.
- [19]. Brusica V, Petrovsky N. Immunoinformatics – the new kid in town. *Novartis Found Symp.* 2003; 254: 3–13.
- [20]. Ahlers JD, Belyakov IM, Thomas EK, Berzofsky JA. High affinity T helper epitope induces complementary helper and APC polarization, increased CTL, and protection against viral infection. *J Clin Invest.* 2001; 108: 1677–1685.
- [21]. De Groot AS, Sbai H, Saint-Aubin C, Mc Murry JA, Martin W. Immunoinformatics: mining genomes for vaccine components. *Immunol Cell Biol.* 2002; 80: 255–269.
- [22]. Inaba H, Martin W, De Groot AS, Qin S, De Groot LJ. Thyrotropin receptor epitopes and their relation to histocompatibility leukocyte antigen-DR molecules in Graves' disease. *J Clin Endocrinol Metab.* 2006; 91: 2286–2294.
- [23]. Shafiq MI, Steinberger T, Schimid R. Fascaplysin as a specific inhibitor for CDK4: Insights from molecular modeling. *PLoS One.* 2012; 7: e42612.
- [24]. Kensler TW, Qian GS, Chen JG, Groopman JD. Ranslational strategies for Cancer prevention in liver. *Nature Publishing Group.* 2003; 3: 321 -329.
- [25]. Lukas B, Simone V, Heike J, Rowena B Jurgen R. Nicotinamide phosphoribosyltransferase and prostaglandin H2 synthase 2 are up-regulated in human pancreatic adenocarcinoma cells after stimulation with interleukin-1. *Inter J of Onco.* 2009; 35: 97-107.
- [26]. Vogelstein B, Sur S, Prives C. p53: The Most Frequently Altered Gene in Human Cancers. *Nature Education.* 2010; 3: 6.
- [27]. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annual Review of Pharmacol Toxicol.* 2005; 45: 51.
- [28]. Eyerman MC, Wysocki L. T cell recognition of somatically generated Ab diversity. *J Immunol.* 1994; 152: 1569–1577.
- [29]. Reveille JD. The genetic basis of autoantibody production. *Autoimmune Rev.* 2006; 5: 389–98.





- [30]. De Groot AS, Martin W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. *Clin Immunol.* 2009; 131:189-201.
- [31]. Baloria U, Akhooon BA, Gupta SK, Sharma S, Verma V. *In silico* proteomic characterization of human epidermal growth factor receptor 2 (HER-2) for the mapping of high affinity antigenic determinants against breast cancer. *Amino Acids.* 2012; 42: 1349–1360.
- [32]. Pedretti A, Villa L, Vistoli G. VEGA – An open platform to develop chemo-Bio-Informatics applications, using plug-in architecture and script programming. *J Comput Aided Mol.* 2004; 18: 167-73.
- [33]. Scofield RH, Warren WL, Koelsch G, Harley JB. A hypothesis for the hla-b27 immune dys regulation in Spondyloarthritis: contributions from enteric organisms, b27 Structure, peptides bound by b27, and convergent evolution. *Proc Natl Acad Sci.* 1993; 90: 9330-9334.
- [34]. Gupta SK, Srivastava M, Akhooon BA, Smita S, Schmitz U, et al., Identification of immunogenic consensus T-cell epitopes in globally distributed influenza-A H1N1 neuraminidase. *Infect Genet Evol.* 2010; 11: 308-319.
- [35]. Sette A, Fikes J. Epitope-based vaccines: an update on epitope identification, vaccine design and delivery. *Curr Opin Immunol.* 2003; 15: 461–70.
- [36]. Calderone CT, Williams DH. An enthalpic component in cooperativity: the relationship between enthalpy, entropy, and non-covalent structure in weak associations. *J Am Chem Soc.* 2001; 123: 6262.
- [37]. Flower DR. Towards *in silico* prediction of immunogenic epitopes. *Trends Immunol.* 2003; 24: 667.
- [38]. Seifert UC, Maranon A, Shmueli JF, Desoutter L, Wesoloski K, et al., An essential role for tripeptidyl peptidase in the generation of an MHC class I epitope. *Nat Immunol.* 2003; 4: 375.
- [39]. Francois PY Lamour, Pilar Lardelli, Apfel CM. Analysis of the ligand-binding domain of human retinoic Acid Receptor a by Site-Directed Mutagenesis. *Mol Cell Biol.* 1996; 10: 5386–5392.
- [40]. Llovet JM, Di Bisceglie AM, Bruix J et al., Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst.* 2008; 21: 698–711.
- [41]. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science.* 2003; 301: 513–517.
- [42]. Xiaojun Yan, Haimin Chen, Xiaoling Lu, Feng Wang, Weifeng Xu, et al., Fascaplysin exert anti-tumor effects through apoptotic and anti-angiogenesis pathways in sarcoma mice model. *Eur J Pharmaceu Sci.* 2011; 43: 251–259.

