Genbinesia 1: 35-43 (2021)
doi: xxxxxxx
http://journal.genbinesia.or.id/

RESEARCH ARTICLE

GENBINESIA
Journal of Biology

Viroinformatics study: polytope mapping of envelope glycoprotein to tackle HIV-2 infection and develop vaccine candidate

Viol Dhea Kharisma[1]*, Muhammad Hermawan Widyananda[1], Arif Nur Muhammad Ansori[2], Rasyadan Taufiq Probojati[3], Ahmad Affan Ali Murtadlo[4], Muhammad Badrut Tamam[5], Adhityo Wicaksono[6], Dora Dayu Rahma Turista[7]

[1]*Department of Biology, Faculty of Mathematic and Natural Sciences, Universitas Brawijaya, Malang, Indonesia*
[2]*Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3]*Department of Agrotechnology, Faculty of Agriculture, Universitas Kadiri, Kediri, Indonesia*
[4]*Computational Virology and Complexity Sciences Research Unit, Division of Molecular Biology and Genetics, Generasi Biologi Indonesia Foundation, Gresik, Indonesia*
[5]*Department of Biology, Faculty of Sciences and Technology, Universitas Muhammadiyah Lamongan, Lamongan, Indonesia*
[6]*Independent Researcher of Biotechnology in Jakarta, Indonesia.*
[7]*Faculty of Teacher Training and Education, Mulawarman University, Samarinda, Indonesia*
**Correspondence: violdhea@student.ub.ac.id*

Received:
14 October 2021
Accepted:
27 October 2021
Published:
3 November 2021

Abstract

Human Immunodeficiency Virus type 2 (HIV-2) has been identified to exhibit an ability to resist antiretroviral administration and many scientists has predicted increases in the pathogenicity of HIV-2. The development of a vaccine against the type 1 virus (HIV-1) infection has reached the phase 3 clinical trial stage, but currently there is no information on the development of a vaccine against HIV-2. Vaccine development to trigger an increase in the coverage of the expansion of protection can be done through B cell polytope. This study aims to provide an important preliminary for the construction of vaccine candidates by identifying the peptides that make up the B cell polytope in the HIV-2 envelope glycoprotein region. The HIV-2 sequence was obtained from the database. The study followed by 3D modelling, prediction of linear B-cell epitope mapping, antigenicity, allergenicity, peptide properties, and immune simulation was carried out via a webserver. The 3D structure of the peptide was displayed through molecular visualization software. The results showed that the 23-mer peptides E1 'HPRYTGVKNIRDITLTEPGRGSD', F1 'NFIENRKGTQHN' 12-mer, M1 'YLKDQARLNS' 10-mer, N1 'PWVNDSIQPNWNNMTWQQWEL QVRD' 25-mer, and O1 'KLQNSWNMGVQTO' can be used as a candidate polytope HIV-2 vaccine because it is recognized by B cells is an antigenic peptide with stable molecule, non-allergenic. The peptides trigger proliferation and activation of B cells to produces a humoral response and work as functionally protective antibody for neutralization of HIV-2.

Keywords: Acquired immune deficiency syndrome, B-cell, bioinformatics, human immunodeficiency virus, retrovirus

Introduction

The first cases of human immunodeficiency virus type 2 (HIV-2) infection were identified in West Africa, affecting 2 million people in 1985, but then the case have also been found in France, Portugal, USA, and India. HIV-2 is predicted to be associated with viruses derived from the primate Sooty Mangabey, in contrast to the type 1 virus (HIV-1) originated from gorillas and chimpanzees[1]. New research reveals that HIV-2 has lower rates of transmission and virulence than HIV-1, but that HIV-2 also triggers acquired immune deficiency syndrome (AIDS). HIV-1 consists of viral groups M, N, O, P, with the M group consists of 12 subtypes and has the highest prevalence rate since the virus was first discovered in 1920. Since 2010, HIV-2 has been identified as having eight groups of AH[2].

HIV-2 has a lower level of pathogenicity than HIV-1 and the mechanism that causes the low virulence of HIV-2 has not been found, and the antiretroviral drugs can be used only in HIV-1[3]. HIV-2 is resistant to non-nucleoside reverse transcriptase inhibitors (NNRTI) antiviral type by a unknow mechanism. The evidence base for HIV-1 and HIV-2 coinfection has not been found, but many scientists predict the possibility of this mechanism because the two viruses can have symbiosis to strengthen the pathogenicity and level of resistance to antiretroviral drugs[4].

The development of HIV vaccines with various types such as DNA, virus-like particles, recombinant subunits, mRNA with clinical trials from phase 1 to 3, has been done since 1999 until present[5]. Research on HIV-1 vaccines such as Uhambo or HVTN 702 from Africa was discontinued in 2020 because they did not have effective efficacy in reducing viral load, but until now there are HIV-1 vaccine candidates such as the mRNA type (eOD-GT8 60mer) developed by Scripps Research & IAVI, Ad26.Mos4.HIV with envelope glycoprotein or viral vector vaccine type and subunit with the aim of increasing protection from global strain infection with HIV-1 strain from 2020 until now currently undergoing phase 3 clinical trial[6]. However, from various information on clinical trials of HIV vaccines, no research information was found aimed at developing an HIV-2 vaccine.

From the development progress of the HIV-1 vaccine to the clinical trial phase 3, there is also a subunit-type vaccine through the envelope glycoprotein, the use of the HIV-2 envelope glycoprotein in this study refers to the previous type of HIV-1 vaccine. Viroinformatics is a research approach that combines the fields of Bioinformatics with Virology to explore solutions that produce a prediction for solving problems related to viruses[7]. The combination of several peptides that make up the epitope that provides an extension of antibody neutralization and recognition of immune cells against pathogens is called a polytope[8]. The viroinformatics approach is possible as a one step in the development of the HIV-2 vaccine, in this study screening of predicted polytope or region to produce an effective vaccine candidate peptide and provide a protective immune response against HIV-2 infection

Materials and methods

Sample Preparation

The HIV-2 envelope protein sequence with ID ADH04368.1 was obtained from the NCBI database (*https://www.ncbi.nlm.nih.gov/*), the sequence consists of three regions, namely envelope, gp120, and transmembrane protein[9]. This study used the entire envelope of the HIV-2 virion, the sequence on the envelope having a length of 830-mer.

Procedures

Protein modelling

The 3D structure of the HIV-2 gp120 envelope model construction and validation were carried out via the SWISS-MODEL server (*https://swissmodel.expasy.org/*). SWISS-MODEL works based on the identification of the similarity of the query sequence with the template to generate model construction. Homologous models were identified with a score of 20% and a validation score of the Ramachandran plot of 80%[10,11].

B-cell linear epitope mapping

Recognition of B cell epitope on HIV-2 gp120 envelope via IEDB Analysis Resource server (*http://tools.iedb.org/bcell/*) using BepiPred 2, Kolaskar-Tongaonkar, and Emini Surfaces Accessibility methods. The B cell epitope probability score was generated by calculating the propensity scale, Hidden Markov Model, and predicting the physicochemical properties of the amino acids that make up the peptide. In the graph, the prediction results show that the peptide with the green area is a negative result as a B cell epitope while yellow is a positive prediction[12].

Antigenicity, allergenicity, and properties

The vaccine candidate peptides that make up the B cell linear epitope are then predicted as antigenic peptides through VaxiJen 2.0 version (*http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html*) and for allergenic peptide prediction using AllerTop 2.0 version (*https: //www.ddg-pharmfac.net/AllerTOP/index.html*). Antigenic and non-allergenic peptides predicted molecular properties with the Exspasy ProtParam Tool (*https://web.expasy.org/protparam/*). The calculations used in ProtParam consist of theoretical pi, aliphatic index, GRAVY, and instability index[13].

Immune simulation of vaccine candidate

Stable peptides were simulated via C-ImmSim (*http://kraken.iac.rm.cnr.it/C-IMMSIM/*). Simulation-based on the ability of vaccine candidate peptides when triggering humoral immunity such as the amount of B cell production and antibody secretion in human. This study uses the default MHC allele with simulation step parameters 1000, 100 volume, Random Seed 12345 on C-ImmSim[14].

Results

The Model Identification of Envelope gp120 HIV-2

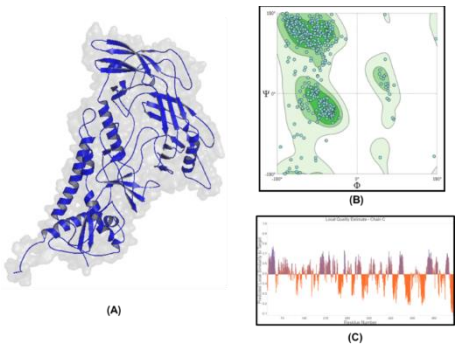

Figure 1. Modeling and validation of the HIV-2 gp120 envelope structure. (A) 3D structure (B) Ramachandran plot (C) Similarity of query sequences and templates.

Prediction of Peptide Vaccine Candidate of HIV-2

Table 1. The result of B-cell linear epitope prediction

B-cell Prediction	Label	Position		Peptide	Length (mer)
		Start	End		
BepiPred 2.0	A1	114	133	INETSSCIRTDSCSGLGNEE	20
	B1	145	158	ERDKIKQYSETWHS	14
	C1	173	187	TSVITESCDKHYWDA	15
	D1	205	222	DTDYSGFEPNCTKVVAAT	18
	E1	327	349	HPRYTGVKNIRDITLTEPGRGSD	23
	F1	373	384	NFIENRKGTQHN	12
	G1	398	408	HKVGQNVYLPP	11
	H1	428	448	WTNITFSAEVAELYRLELGDY	21
	I1	455	473	PIGFAPTSQKRYSSAPGRG	19
	M1	549	558	YLKDQARLNS	10
	N1	572	596	PWVNDSIQPNWNNMTWQQWELQVRD	25
	O1	612	628	QEKNMYELQKLNSWGVF	17
Emini Surfaces Accessibility	A2	144	158	LERDKIKQYSETWHS	15
	B2	375	384	IENRKGTQHN	10
	C2	460	469	PTSQKRYSSA	10
	D2	608	622	AQIQQEKNMYELQKL	15
	E2	689	704	HTDRDQPAREETEEDV	16
Kolaskar-Tongaonkar	A3	19	30	KDVICGRCYMSH	12
	B3	50	63	FRYCAPPGFALLRC	14
	C3	365	384	RTLLAGIVQQQQQLLDVVKR	20
	D3	421	434	CAFRQVCHTTVPWV	14
	E3	495	525	TSWVSYIRYCRVYIIAGVVALRIVIYILQML	31
	F3	533	548	RPVFSSPPGYIQQIHI	16
	G3	576	601	PIAYIHFLIRLLIRLLTGLYNICRGL	26
	H3	603	622	SRSFPILQPIFQSLQRALTA	20
	I3	629	639	LKAAFLQYGCE	11

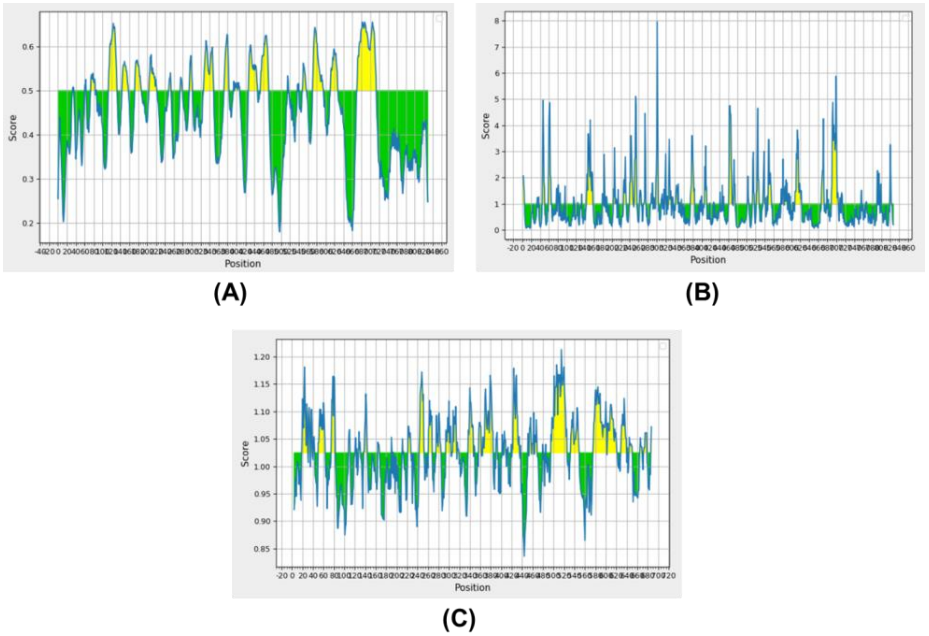

Figure 2. Plot prediction of B cell linear epitope on HIV-2 gp120 envelope through three methods. (A) BepiPred 2 (B) Emini Surfaces Accessibility (C) Kolaskar-Tongaonkar.

Table 2. Antigenicity and allergenicity prediction results on HIV-2 vaccine peptides

Label	Peptide	Antigenicity	Allergenicity
A1	INETSSCIRTDSCSGLGNEE	Antigen	Allergen
B1	ERDKIKQYSETWHS	Non-antigen	-
C1	TSVITESCDKHYWDA	Non-antigen	-
D1	DTDYSGFEPNCTKVVAAT	Antigen	Allergen
E1	HPRYTGVKNIRDITLTEPGRGSD	Antigen	Non-allergen
F1	NFIENRKGTQHN	Antigen	Non-allergen
G1	HKVGQNVYLPP	Non-antigen	-
H1	WTNITFSAEVAELYRLELGDY	Antigen	Allergen
I1	PIGFAPTSQKRYSSAPGRG	Antigen	Non-allergen
M1	YLKDQARLNS	Antigen	Non-allergen
N1	PWVNDSIQPNWNNMTWQQWELQVRD	Antigen	Non-allergen
O1	QEKNMYELQKLNSWGVF	Antigen	Non-allergen
A2	LERDKIKQYSETWHS	Non-antigen	-
B2	IENRKGTQHN	Antigen	Allergen
C2	PTSQKRYSSA	Antigen	Non-allergen
D2	AQIQQEKNMYELQKL	Non-antigen	-
E2	HTDRDQPAREETEEDV	Non-antigen	-
A3	KDVICGRCYMSH	Antigen	Non-allergen
B3	FRYCAPPGFALLRC	Antigen	Non-allergen
C3	RTLLAGIVQQQQQLLDVVKR	Antigen	Non-allergen
D3	CAFRQVCHTTVPWV	Antigen	Allergen
E3	TSWVSYIRYCRVYIIAGVVALRIVIYILQML	Non-antigen	-
F3	RPVFSSPPGYIQQIHI	Non-antigen	-
G3	PIAYIHFLIRLLIRLLTGLYNICRGL	Non-antigen	-
H3	SRSFPILQPIFQSLQRALTA	Non-antigen	-
I3	LKAAFLQYGCE	Antigen	Allergen

Table 3. The result of peptide properties identification

Label	Peptide	Theoretical Pi	Aliphatic Index	GRAVY	Instability Index
E1	HPRYTGVKNIRDITLTEPGRGSD	8.60	63.48	-1.139	Stable
F1	NFIENRKGTQHN	8.75	32.50	-1.908	Stable
I1	PIGFAPTSQKRYSSAPGRG	11.00	31.05	-0.837	Unstable
M1	YLKDQARLNS	8.59	88.00	-1.160	Stable
N1	PWVNDSIQPNWNNMTWQQWELQVRD	4.03	54.40	-1.308	Stable
O1	QEKNMYELQKLNSWGVF	6.14	62.94	-0.924	Stable
C2	PTSQKRYSSA	10.01	10.00	-1.610	Unstable
A3	KDVICGRCYMSH	8.05	56.67	-0.167	Unstable
B3	FRYCAPPGFALLRC	8.96	70.00	0.564	Unstable
C3	RTLLAGIVQQQQQLLDVVKR	10.84	146.00	-0.045	Unstable

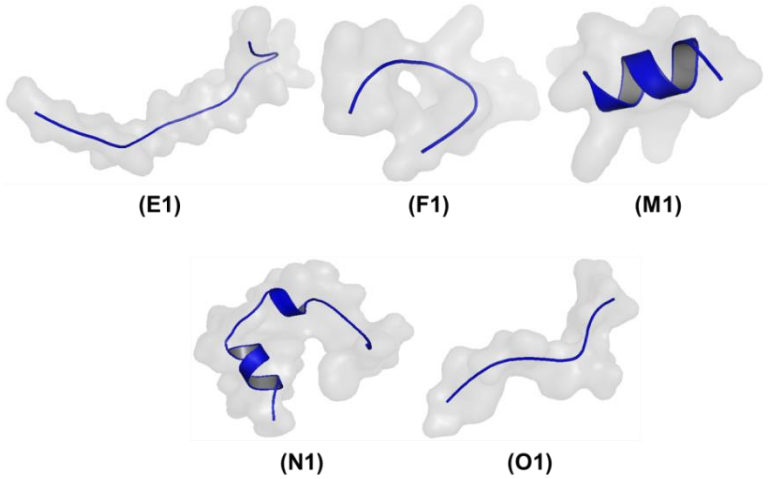

Figure 3. HIV-2 vaccine candidate peptide model visualization. Blue is used for cartoons while gray is for transparent surfaces.

Immune Simulation of HIV-2 Vaccine Candidate

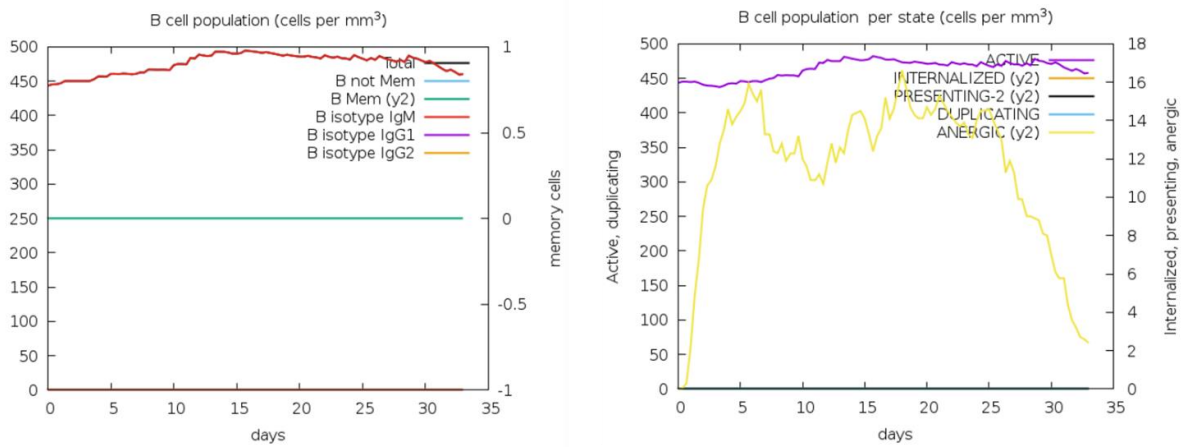

Figure 4. B-cell polytope immune simulation of HIV-2 peptide vaccine candidate.

Discussion

The Model Identification of Envelope gp120 HIV-2

The 3D structure construction is carried out using the homology modelling method, the model with a similarity value of 20% is homologous to the template[15,16]. The homologous protein model has an abundance of conserved regions and allows 85% to 90% to be close to accurate, then the model is validated through the Ramachandran plot with consideration of a score of 80% for the favored region[17]. The results showed that the HIV-2 gp120 envelope protein modelling sequence (ID ADH04368.1) had a similarity of 37.04% and a score of 87.85% favored region and 0% bad bond. Visualization of the 3D envelope gp120 HIV-2 model was performed using EduPyMol software with cartoons structure and transparent surfaces (Figure 1).

Prediction of Peptide Vaccine Candidate of HIV-2

The HIV-2 envelope glycoprotein allows it to be recognized as an epitope and triggers B cell activation via a direct pathway. B cell epitope prediction using the BepiPred 2 method works based on the Random Forest algorithm calculation with a threshold of 0.500 to determine the probability of the epitope

in the antigen query sequence[18]. The Emini Surfaces Accessibility method is used to predict B cell epitopes by calculating the probability of amino acids on the surface of the query antigen sequence with 1,000 as the threshold[19]. Prediction of B cell epitope through the Kolaskar-Tongaonkar method based on the calculation of the physicochemical probability of each amino acid making up the epitope with an accuracy of 75%[13]. The results showed that the peptides obtained from the predicted B cell epitope were twelve with label 1, five for peptides from Emini Surfaces Accessibility with label 2, and nine for peptides from Kolaskar-Tongaonkar with label 3, the total number of all peptides was twenty-six. with different lengths and positions on the HIV-2 gp120 envelope (Table 1). Positive predictions with the plot region being above the threshold as the epitope of cell B are shown in the graph in yellow and negative or the region is below the threshold (Figure 2).

Epitope composing peptides derived from positive predictions of the three methods consisting of BepiPred 2, Emini Surfaces Accessibility, and Kolaskar-Tongaonkar were identified as antigenic peptides through VaxiJen. The server works in determining antigen scores by calculating cross-covariance (ACC) on protein sequences with an accuracy of about 70% to 89%, the target organisms consist of bacteria, viruses, and tumors[20,21]. Then identification of allergenicity was carried out on antigenic peptides through AllerTop using the ACC method to explain the physicochemical properties of the amino acid composition to determine the categories of allergens and non-allergens. The results of this study show (Table 2).

Antigenic peptides with non-allergenic properties are identified through PortParam, as this server is used to calculate chemical and physical parameters on vaccine candidate peptides. The parameters used in this study are theoretical pi, aliphatic index, grand average of hydropathicity (GRAVY), and instability index[22]. The results of this study showed that there were five stable vaccine candidate peptides (Table 3.), then the 3D structure of the peptides was displayed using EduPyMol software with cartoons structure and transparent surfaces with publication standard staining (Figure 3).

In the end, five peptides, 23-mer peptides E1 'HPRYTGVKNIRDITLTEPGRGSD', F1 'NFIENRKGTQHN' 12-mer, M1 'YLKDQARLNS' 10-mer, N1 'PWVNDSIQPNWNNMTWQQWELQVRD' 25-mer, and O1 'KLQNSWNMGVQTO', appear to possess promising result, as they are recognizable by B-cells as the antigenic peptides (Table 1), non-allergenic (Table 2), and with stable molecular structure (Table 3). These peptides are able to trigger proliferation and activation of B cells, allowing production of humoral responses, and hence are functional as protective antibodies for neutralizing HIV-2.

Immune Simulation of HIV-2 Vaccine Candidate

When the HIV-2 viral entry mechanism occurs in host cells, the envelope glycoprotein or gp120 has an important role for attachment to the CD4+ receptor[23]. The design of the HIV-2 vaccine candidate with identification of the epitope on gp120 allows for the construction of antigenic peptides that trigger B cell recognition and immune response. The results showed that a polytope consisting of peptides E1, F1, M1, N1, and O1 could trigger the proliferation of B cell clones with IgM antibody isotypes and the activation process with a total population of 450 cells per mm3 (Figure 4). Polytope can be categorized as a good vaccine candidate because it can trigger a humoral response to neutralize HIV-2 infection.

Conclusions

The 23-mer peptides E1 'HPRYTGVKNIRDITLTEPGRGSD', F1 'NFIENRKGTQHN' 12-mer, M1 'YLKDQARLNS' 10-mer, N1 'PWVNDSIQPNWNNMTWQQWELQVRD' 25-mer, and O1 'KLQNSWNMGVQTO' HIV vaccine can be used 2 because it is recognized by B cells as antigenic peptides, molecularly stable, non-allergenic, trigger proliferation and activation of B cells to produce humoral responses and are functionally protective antibodies for neutralizing HIV-2.

Acknowledgments

This study was supported by a research grant from the Division of Molecular Biology and Genetics, Generasi Biologi Indonesia Foundation, Indonesia. We thank EJA Team, Indonesia for editing the manuscript.

Conflicts of Interest

Any potential conflicts of interest are noted.

References

1. Esbjörnsson J, Jansson M, Jespersen S, et al. HIV-2 as a model to identify a functional HIV cure. *AIDS Res Ther*. Published online 2019. doi:10.1186/s12981-019-0239-x
2. Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. *Clin Infect Dis*. Published online 2011. doi:10.1093/cid/ciq248
3. Nsagha DS, Njunda AL, Kamga HLF, Assob JCN, Bongkem EA. HIV-1/HIV-2 co-infection among voluntary counselling and testing subjects at a regional hospital in Cameroon. *Afr Health Sci*. Published online 2012. doi:10.4314/ahs.v12i3.5
4. Raugi DN, Nixon RS, Leong S, et al. HIV-2 drug resistance genotyping from dried blood spots. *J Clin Microbiol*. Published online 2021. doi:10.1128/JCM.02303-20
5. Lee JH, Crotty S. HIV vaccinology: 2021 update. *Semin Immunol*. Published online 2021. doi:10.1016/j.smim.2021.101470
6. Baden LR, Stieh DJ, Sarnecki M, et al. Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. *Lancet HIV*. Published online 2020. doi:10.1016/S2352-3018(20)30229-0
7. Agrawal L, Poullikkas T, Eisenhower S, et al. Viroinformatics-Based Analysis of SARS-CoV-2 Core Proteins for Potential Therapeutic Targets. *Antibodies*. Published online 2021. doi:10.3390/antib10010003
8. Pouyanfard S, Spagnoli G, Bulli L, et al. Minor Capsid Protein L2 Polytope Induces Broad Protection against Oncogenic and Mucosal Human Papillomaviruses. *J Virol*. Published online 2018. doi:10.1128/jvi.01930-17
9. Kharisma VD, Ansori ANM, Posa GAV, Rizky WC, Permana S, Parikesit AA. Conserved B-cell epitope identification of envelope glycoprotein (GP120) HIV-1 to develop multi-strain vaccine candidate through bioinformatics approach. *J Teknol Lab*. Published online 2021. doi:10.29238/teknolabjournal.v10i1.274
10. Hartati FK, Djauhari AB, Viol Dhea K. Evaluation of pharmacokinetic properties, toxicity, and bioactive cytotoxic activity of black rice (Oryza sativa l.) as candidates for diabetes mellitus drugs by in silico. *Biointerface Res Appl Chem*. Published online 2021. doi:10.33263/BRIAC114.1230112311
11. Fahmi M, Kharisma VD, Ansori ANM, Ito M. Retrieval and Investigation of Data on SARS-CoV-2 and COVID-19 Using Bioinformatics Approach. In: *Advances in Experimental Medicine and Biology*. ; 2021. doi:10.1007/978-3-030-63761-3_47
12. Fadholly A, Ansori ANM, Kharisma VD, Rahmahani J, Tacharina MR. Immunobioinformatics of rabies virus in various countries of asia: Glycoprotein gene. *Res J Pharm Technol*. Published online 2021. doi:10.5958/0974-360X.2021.00157.8
13. Rantam FA, Kharisma VD, Sumartono C, et al. Molecular docking and dynamic simulation of conserved B cell epitope of SARS-CoV-2 glycoprotein Indonesian isolates: An immunoinformatic

approach. *F1000Research*. Published online 2021. doi:10.12688/f1000research.54258.1
14. Banerjee S, Majumder K, Gutierrez GJ, Gupta D, Mittal B. Immuno-informatics approach for multi-epitope vaccine designing against SARS-CoV-2. *bioRxiv*. Published online 2020. doi:10.1101/2020.07.23.218529
15. Widyananda MH, Pratama SK, Samoedra RS, et al. Molecular docking study of sea urchin (Arbacia lixula) peptides as multi-target inhibitor for non-small cell lung cancer (NSCLC) associated proteins. *J Pharm Pharmacogn Res*. Published online 2021.
16. Prahasanti C, Nugraha AP, Kharisma VD, et al. A bioinformatic approach of hydroxyapatite and polymethylmethacrylate composite exploration as dental implant biomaterial. *J Pharm Pharmacogn Res*. Published online 2021.
17. Luqman A, Kharisma VD, Ruiz RA, Götz F. In silico and in vitro study of Trace Amines (TA) and Dopamine (DOP) interaction with human alpha 1-adrenergic receptor and the bacterial adrenergic receptor QseC. *Cell Physiol Biochem*. Published online 2020. doi:10.33594/000000276
18. Purnama ER, Kharisma VD. Epitope Mapping of Capsid Protein L1 from Human Papillomavirus to Development Cervical Cancer Vaccine through Computational Study. In: *Journal of Physics: Conference Series*. ; 2018. doi:10.1088/1742-6596/1108/1/012096
19. Ansori ANM, Kharisma VD, Antonius Y, Tacharina MR, Rantam FA. Immunobioinformatics analysis and phylogenetic tree construction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Indonesia: spike glycoprotein gene. *J Teknol Lab*. Published online 2020. doi:10.29238/teknolabjournal.v9i1.221
20. Adianingsih OR, Kharisma VD. Study of B cell epitope conserved region of the Zika virus envelope glycoprotein to develop multi-strain vaccine. *J Appl Pharm Sci*. Published online 2019. doi:10.7324/JAPS.2019.90114
21. Doytchinova IA, Flower DR. VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*. Published online 2007. doi:10.1186/1471-2105-8-4
22. Pan W, Chen D-S, Lu Y-J, et al. Bioinformatic prediction of the epitopes of Echinococcus granulosus antigen 5. *Biomed Reports*. Published online 2017. doi:10.3892/br.2016.827
23. Wilen CB, Tilton JC, Doms RW. HIV: Cell binding and entry. *Cold Spring Harb Perspect Med*. Published online 2012. doi:10.1101/cshperspect.a006866