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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

The term "glioma" refers to a primary malignant brain tumour. Mutation of isocitrate dehydrogenase type 1 (IDH1) can be a marker for the presence of glioma. IDH1 (R132H) specific immunogenic epitopes can be employed as a glioma vaccination. Homology modelling of 91 epitopes of IDH1 (R132H) that have the possibility of being an antigen has been performed. The aims of this study are to predict the greatest preventative and curative activity of the epitopes through docking study and to predict stability of the epitope-receptor complex through molecular dynamics simulation. The glioma prevention function of the epitopes can be predicted by docking the epitopes to major histocompatibility complexes class II (MHC II). Meanwhile, its curative function was predicted by docking the epitopes on the ephrin type-A receptor 3 (EphA3). It was accomplished with Dock version 6.7. The method of docking utilised in this investigation was rigid body docking. The highest binding affinity for MHC II of all the test epitopes is indicated by epitope 42, as measured by a grid score of -62.73 kcal/mol. Epitope 54, with a grid score of -55.56 kcal/mol, has the highest binding affinity for the EphA3 receptor. The epitope 42-MHC II complex proved unstable in GROMACS version 5.0.6 molecular dynamics simulations at 300 K for 25 ns. The epitope 54-EphA3 complex, on the other hand, remained steady from the start of the simulation through 15.29 ns. The best candidate for a prophylactic glioma vaccination is epitope 42, and the best candidate for a curative glioma vaccine is epitope 54.

Keywords: epitopes, glioma, IDH1 (R132H), in silico, vaccine

1. Introduction

Brain tumours encompass roughly 85-90% of all central nervous system cancers. The most prevalent type of brain tumour is a glial cell tumour known as a glioma (KPKN, 2015). Glioma is a malignant primary brain tumour. Numerous efforts have been made to determine the genetic basis of these tumours in the hope that the knowledge gained will aid in developing therapy with higher targets to improve the prognosis of patients with a poor prognosis (Dimitrov et al., 2015).

The body uses an enzyme called isocitrate dehydrogenase 1 (IDH1) to generate adenosine triphosphate (ATP) in the citric acid cycle. Mutations in IDH1 can result in the production of an oncometabolite, 2-hydroxyglutarate (Schumacher et al., 2014a; Turcan et al., 2012; Yang et al., 2010). In a limited number of glioblastomas, a somatic mutation in codon 132 of the IDH1 gene at locus chromosome 2q33 has been identified. Nonetheless, this mutation has been discovered in a lot of low-grade gliomas. (Balss et al., 2008; Bleeker et al., 2010; Cohen et al., 2013; Labussiere et al., 2010). There are six different mutations of IDH1, with the largest

frequency being R132H mutations in which arginine transforms into histidine (89.7%) (Arita et al., 2015). Therefore, IDH1 (R132H) can be a marker of a glioma (Labussiere et al., 2010).

Due to its potential as a tumour-specific neoantigen, IDH1 (R132H) is a potential immunotherapy target. IDH1 (R132H) possesses immunogenic epitopes that are suitable for vaccination. IDH1 (R132H) epitopes are presented in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with IDH1 (R132H) mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially. All tumour cell surfaces exhibit IDH1 in its R132H form, which is unique to the tumour. Thereby, vaccines can alert the patient's immune system to IDH1 (R132H) without causing harm to the healthy cells (Archer et al., 2014; Pellegatta et al., 2015; Schumacher et al., 2014b).

Based on their intended use, there are two types of cancer vaccines. Prophylactic cancer vaccines are employed to prevent cancer, and therapeutic cancer vaccines are applied to treat the condition and build bodily resistance to cancer (Lollini et al., 2006). Antigenic peptides are generated from proteins produced by tumour cells of interest. For the development of peptide-based vaccines, it is crucial to predict whether peptides will bind to specific MHC molecules in humans. A computational method has been developed to identify these peptides (Schiewe and Haworth, 2007). Utilisation of in silico-based methods with which to predict epitopes for producing peptide vaccines that have been designed rationally can improve the efficacy of the vaccine (Cherryholmes et al., 2015).

Docking studies, which dominated computer-aided drug design (CADD), are undertaken for virtual screening or lead optimization in drug screening and design. Protein-ligand or protein-protein docking studies can predict the direction of a ligand when it is bound to a protein receptor or enzyme (Chen, 2015). The molecular dynamics simulation is utilized to comprehend the physical underpinnings of the structure and the function of biological macromolecules. In molecular dynamics simulation, proteins have a dynamic model in which internal motions and conformational changes are crucial to their function (Karplus and McCammon, 2002). The root mean square deviation (RMSD) graph presents a sharp slope for the first few nanoseconds (ns) and oscillates around a constant average value for the rest of the simulation. The root mean square fluctuations (RMSF) graph shows the magnitude of fluctuations of every atom or residue in the protein (Abraham et al., 2015).

Homology modelling of 91 epitopes of IDH1 (R132H) that have the possibility of being a cancer antigen has been performed, validated and refined (Yeni and Tjahjono, 2017). Based on these matters, the aims of this study are to find the greatest preventative and curative activity of the epitopes as a glioma vaccine and to predict stability of the epitope-receptor complex. Some docking study and molecular dynamics simulation devices were utilised to determine the affinity of epitopes against MHC II for predicting preventative activity and to ephrin type-A receptor 3 (EphA3) receptors for predicting curative activity.

2. Methodology

2.1 Docking Studies

The docking study was conducted using Dock version 6.7 (Theresa et al., 2015). IDH1 (R132H) epitopes (Table 1) are docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the

EphA3 (PDB: 4TWO) receptor, which has been separated by their native ligand using Discovery Studio version 16.1.0.15350. The structure of the receptors was obtained from the Protein Data Bank (http://rcsb.org/). The native ligand of MHC II is the A2 peptide, and the native ligand EphA3 is that of compound 164. The docking method used in this study is rigid body docking (Mohan et al., 2005; Vail et al., 2014). Redocking between the receptors and their native ligand is performed before docking epitopes to the receptors to obtain RMSD ≤ 2 Å (Kiszka, 2011).

Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccine

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS
20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI
21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitopes and receptors were prepared by adding hydrogen and charge, then surface manufacture using Chimera version 1.10.2. Spherical epitopes and receptors were then formed to obtain several clusters. One cluster of the receptors with the greatest number of spheres with native ligands is selected. After that, a box is created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid and then redocking. The redocking grid is also used for docking epitopes to receptors. The grid score is obtained from the docking results. A more negative grid score means a greater affinity of the epitope-receptor bond. Docking results were visualised using Discovery Studio. The epitope with the most negative grid score was chosen for molecular dynamics simulation.

2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015), with a temperature of 300 K for the epitope-MHC II and epitope-EphA3 complexes selected in the previous stage. In the molecular dynamics simulation, the LINCS algorithm is used in the AMBER99SB-ILDN force field. The simulation was performed for 25 ns. The structural changes can be analysed from the value of RMSD. Visualisation of molecular dynamics simulations can use VMD version 1.9.2 (Humphrey et al., 1996; Mathews, 2009).

3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands established in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligand of MHC II is peptide A2, and the native ligand of EphA3 is compound 164. Results of redocking comprised the RMSD value for MHC II with the A2 peptide (0.7371 Å) and for receptor EphA3 with compound 164 (1.2801 Å). The RMSD values are <2 Å. It suggests that the method can be used for virtual screening using epitopes.

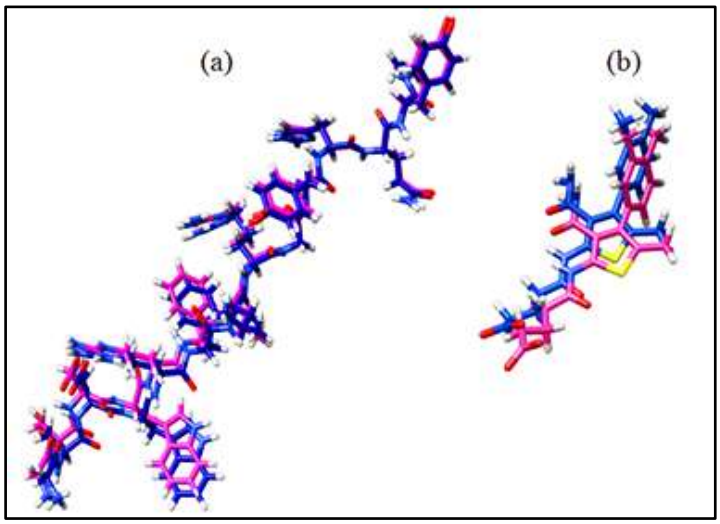

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a) and native ligands EphA3, compound 164 (b)

The docking study was conducted on a 3D structure of 91 epitopes to find their activity concerning receptors. The docking method is the rigid body docking method, wherein the epitope or ligand and receptor are treated in a rigid state.

The epitope activity for glioma prevention is known from docking results concerning MHC II (Table 2). The epitope activity for glioma treatment is known from the docking results of the EphA3 receptor (Table 3). The grid score is obtained in the docking results. The grid score equals the sum of the Van der Waals and electrostatic energies. The more negative the grid score, the stronger the connection between the epitope and the receptor.

Table 2. Results of epitope docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitope docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of epitope docking with MHC II obtained the most negative grid score on epitope 42 (-62.73 kcal/mol with seven hydrogen bonds) (Figure 2a). Although the grid score was not more negative than the grid score of redocking the A2 peptide to MHC II, epitope 42 remains likely to be a new candidate prophylactic vaccine for gliomas because the A2 peptide is an endogenous peptide used in this study only to find the active side of MHC II (Murthy and Stern, 1997; Wang et al., 2010, 2008). Meanwhile, the results of epitope docking with the EphA3 receptor witnessed the most negative grid score on epitope 54 (-55.56 kcal/mol with 11 hydrogen bonds) (Figure 2b). The grid score is more negative than the grid score for redocking compound 164 to the EphA3 receptor.

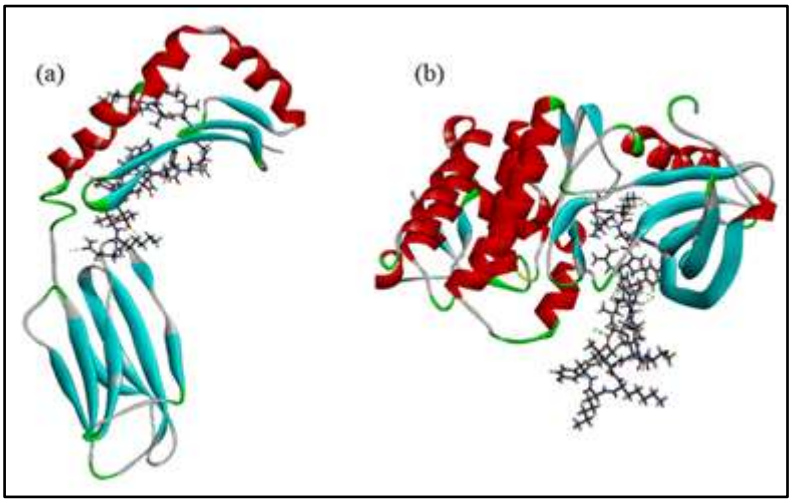

Figure 2. Visualisation of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on the epitope 42-MHC II and epitope 54-EphA3 receptor complexes. In the molecular dynamics simulation, the epitope and receptor are flexible (Ciccotti et al., 2014; Oelmeier et al., 2012). This simulation aims to determine the

stability of docked epitope-receptor complexes. Simulation of molecular dynamics was performed for 25 ns.

The stability of the epitope-receptor complex can be analyzed from changes in structural conformation during the molecular dynamics simulation. It can be seen from the RMSD function to simulation time. The simulation of the epitope 42-MHC II complex resulted in drastically increased RMSD at the early stage of the simulation, which was 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the RMSD of the complex decreases to about 4-8 Å. Even though the RMSD decreased, RMSD > 3 Å showed that the complex was unstable during the simulation time (Figure 3). Figure 4 shows the complex conformation changes of the epitope 42-MHC II complex during the simulation.

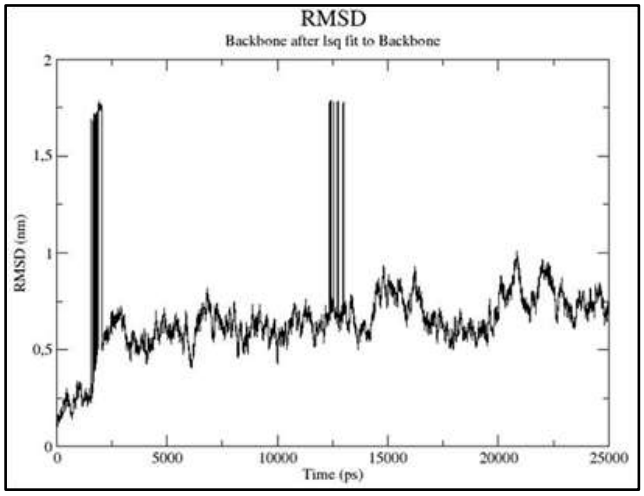

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

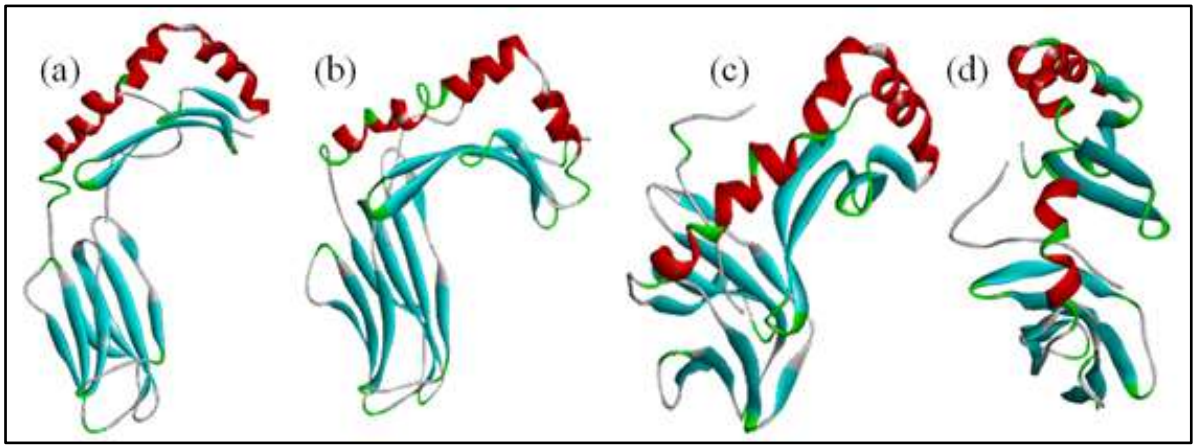

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c) and time 25 ns (d)

In the molecular dynamics simulation of the epitope 54-EphA3 receptor complex, the RMSD fluctuations were stable from the initial simulation until 15.29 ns. Then RMSD increased drastically to 17 Å and remained stable at 15.48 ns. RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å (Figure 5). At 15 ns in the simulation, the 3D form of epitope

54 changed from a coil to a β-sheet on the last seven amino acids: Asn, Thr, Ile, Leu, Lys, Tyr, and Asp (Figure 6).

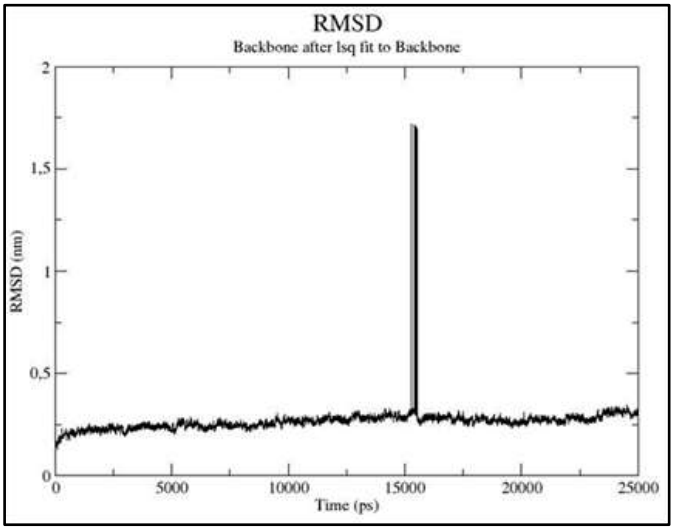

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

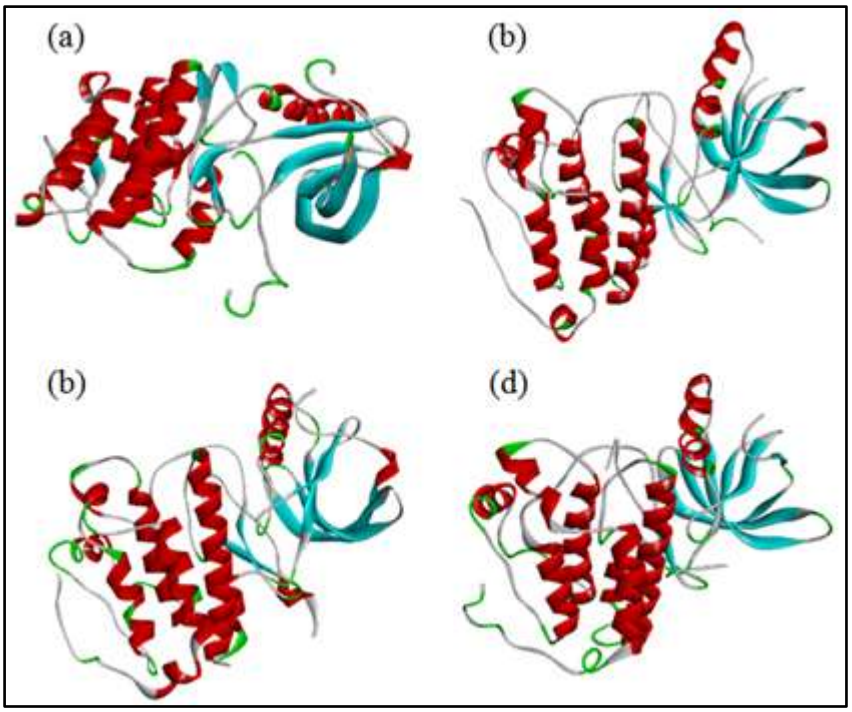

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c) and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes can be analyzed from RMSF values during the simulation (Figure 7). In the simulation, more atoms of the epitope fluctuate than receptors. Atoms of epitope 42 begin from the 3013th atomic order of the complex. Meanwhile, atoms of epitope 54 begin from the 4450th atomic order of the complex.

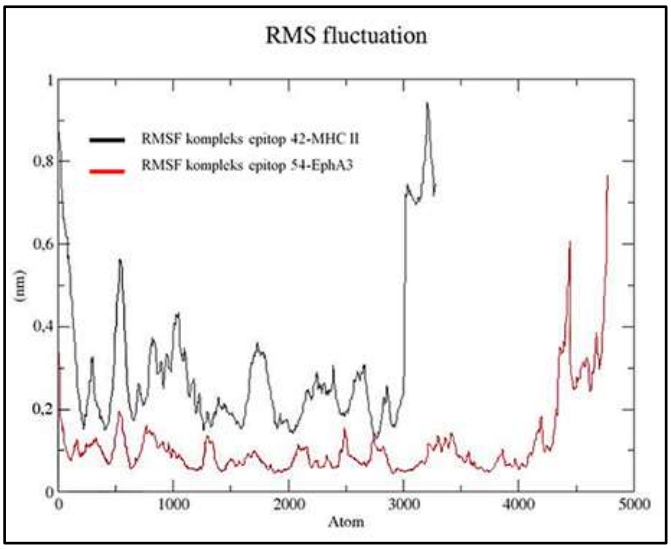

Figure 7. RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The greatest glioma prophylactic vaccine among 91 epitopes of IDH1 (R132H) is epitope 42, while the greatest therapeutic vaccine is epitope 54. The grid score of epitope 42 docking into MHC II is -62.73 kcal/mol. The grid score of epitope 54 docking into the EphA3 receptor is -55.56 kcal/mol. In the molecular dynamics simulation with a temperature of 300 K, the epitope 42-MHC II complex was unstable during the simulation, and the epitope 54-EphA3 receptor complex was stable from the initial simulation until 15.29 ns. It is necessary to synthesize epitope 42 and epitope 54 as well as further experimental testing in vitro with the Hs 683 cell line and in vivo to prove that the epitopes of IDH1 (R132H) have preventive and curative activity against glioma.

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2
Bukti pesan ke tim editor untuk permintaan informasi progress artikel
(23 Desember 2023)

Request information about the progress of article publication

Participants Edit

Mycel A. Capilayan (mjstustp)

Yeni Yeni (yenikind)

Messages

Note	From
Dear MJST Team, We would like to ask about the progress of our manuscript. Hopefully, it will be well received. Best regards, Yeni Yeni	yenikind Dec 23

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3
Bukti konfirmasi review dan hasil review pertama
(15 Januari 2024)

Uhamka
UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMKA

Yeni Uhamka Klender <yeni@uhamka.ac.id>

Double-blind Review Result

1 message

USTP MJST <mjst@ustp.edu.ph> Mon, Jan 15, 2024 at 1:42 PM
To: "yeni@uhamka.ac.id" <yeni@uhamka.ac.id>

Dear Author,

Greetings!

We hope this email finds you well.

This is to inform you that we are done with the double-blind review process for your manuscript entitled "**In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes**". Please log in to your account on our online submission system to check the attached double-blind review results.

Please acknowledge receipt of this email.

Thank you.

Respectfully yours,

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0	01.04.2021	1 of 7

PAPER EVALUATION RESULTS

Paper Code : ECBE1237
Paper Title : In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes
Author(s) : Yeni Yeni

PAPER PROFILE

	First Reviewer	Second Reviewer	Third Reviewer
Originality of the Work	Good	Acceptable	Acceptable
Significant Contribution to the Field	Acceptable	Acceptable	Acceptable
Completeness	Good	Poor	Acceptable
Citation to Other Authors	Acceptable	Poor	Good
Organization of the Manuscript	Excellent	Marginal	Good
Clarity in Writing, Tables, Graphs & Illustrations	Good	Acceptable	Marginal

RECOMMENDATION

First Reviewer	**Acceptable with Revision**
Second Reviewer	**Acceptable with Revision**
Third Reviewer	**Acceptable with Revision**

Note:
*A **paper acceptable with revision** requires changes that would aid in presenting the information more clearly. Further review may be required.*

REVIEWERS' REMARKS

Those suggestions which would improve the quality of the paper but are not essential for publication.

First Reviewer:

1. Overall the manuscript is well-written and well organized.
2. Technical-sounded

Second Reviewer:

1. The author performed a molecular docking analysis of several vaccination candidate epitopes targeting MCH2 and EphA3R. Several points require clarification, as outlined below:

1.1 What was the rationale behind

Third Reviewer:

(none)

	conducting rigid docking in this study? Kindly provide further clarification. *1.2* The author fails to nprovide an explanation of the process of selecting candidate epitopes for docking. *1.3* Prior to conducting molecular docking and dynamics experiments, the author should first make predictions regarding the immunogenicity and allergenicity of the epitope. *1.4* The authors exclude any discussion of their research findings and fail to make any comparisons with prior studies. *1.5* The study lacks a clear delineation of its limitations.	
B. ***Changes which should be made before publication.***		
First Reviewer: 1. Should clarify about the problem/motivation that motivates this work and differentiate this work with existing studies. 2. The references are kind of dated, should be updated with recent reference to keep the work relevant.	Second Reviewer: 1. This paper presents the range expansion of Florida wax Scale (FWS) as affected by Local agricultural trade and climate change. The reviewer finds the paper having a profound contribution to the body of knowledge. The reviewer also finds	Third Reviewer: 1. The manuscript on identification of high-affinity epitopes for glioma is a simple and straightforward application of, off the shelf computational methods. The manuscript in the present form have several issues to be addressed, other than language issues. It should be thoroughly revised before it is accepted for publication. Comments are as follows: (1) In the introduction section. last paragraph – "Homology modelling of 91 epitopes of IDH1 (R132H) … for predicting curative activity." For the benefit of readers, here authors need to provide at least some details of rationale behind selecting 91 epitopes, which were previously published in a non-English journal elsewhere (Yeni and Tjahjono,

	this ecological niche modelling paper very timely and relevant as we are facing the climate change and its inevitable effects. This paper presents and discuss clearly how bioclimatic and anthropocentric factors (i.e. Local Agricultural Trade) could affect the distribution and expansion of FWS in the current situation and future situation. This study could also be done for other taxa of economic importance. Further, this could be done as well for species which are forest dependent or are considered bioindicators as they are the species which are greatly affected by the high rate of habitat destruction nowadays. Based on the conclusion provided, this paper could be a basis in any decision making in the future based on the predicted scenario. The reviewer also commends the authors for providing	2017). In the absence of that, it is hard for a reader to learn about selection of 91 peptide sequences. Moreover, all the reader of this journal are not specialized in immunology, therefore authors should also explain, about using MHC II and EphA3 receptors for predicting preventative and curative activity, resepctively. (2) In abstract "The epitope 42-MHC II complex proved unstable in GROMACS..." is a vague statement. Are they interpreting a conformational transition around 12ns, as a measure of instability? Authors should also need to check the exact cause of large fluctuations at the early stage of simulation in 42-MHC complex (Fig.3). The possible reason could be, either improper minimization, preparation of structural complex, parameter issues etc. It is advisable to redo the simulation. (3) Pl describe in the method section about nature of DOCK grid score. Also explain why none of epitope in Table 2 has significantly lower score than the control A2 peptide. Also it is advisable to evaluate the ranking of protein-peptide complexes using MM-GBSA (or PBSA) Scoring. (4) The language of the manuscript has several issues of improper use of technical words, grammar etc.. It should be thoroughly revised. Some of them are mentioned in the following lines. (5) In abstract and other places in manuscript– "…the greatest preventative and curative activity…" I guess more appropriate usage would be "…high-affinity epitopes as a plausible candidates of preventive and curative activity…". There is a difference between binding affinity and activity. (6) In abstract - "The glioma prevention function of the epitopes can be predicted." Authors through computational methods are attempting prediction of binding affinity and stability of receptor-ligand interaction (and not function prediction). (7) In the method section, "During the docking process, epitopes and receptors were prepared by adding hydrogen and charge, then surface manufacture using Chimera…" Please redraft it.

	recommendations in order to manage the pests and for providing perspective on what other researchable areas should be explored. Overall the reviewer finds the paper very well written and an excellent addition to the body of knowledge. The reviewer is recommending this paper for publication in the journal. However, the reviewer has very few comments and suggestions for the improvement of the manuscript before publication. 2. The reviewer has seen a sentence in the introduction where a period is written before the citation. Period should be removed before the citation in the text (.... And outbreak. (Wan and Yang, 2015)). But I think this was just typographical error, but needs to be corrected. Please check whole manuscript for the same typo error. 3. The authors mentioned at the end part of the	Manufacturing of what? As a general suggestion, I would also advise authors to use prevalent technical words. (8) In Sec 2.2:- Correct the grammar of the following sentence – "Visualisation of molecular dynamics simulations can use VMD…". (9) In Sec 3.1: "…the stronger the connection between the epitope and the receptor." Replace "Connection" to either "binding" or "interaction." (10) In introduction - "Some docking study and molecular dynamics simulation devices were utilised …" Replace "devices" to "methods" and also "Some docking study" with "Docking" (11) Add correct citation of GROMACS as mentioned in - https://doi.org/10.5281/zenodo.10017699

	introduction that there are the five species of Ceroplastes known to occur in the Philippines. The reviewer suggests that the authors should mention these five species in the text. 4. The initial taxonomic identification of the wax scales being controversial was reinforced with whole genome sequencing and its identity was confirmed as C. floridensis (DA-BPI-National Plant Quarantine Services Division, personal communication, August 26, 2021)- Accession code should be cited in the paper. 5. The photo of the FWS in figure 1 is not of the best quality. This could be improved by adding a much clearer photo/s. 6. Figure 6 should be labelled with “a or b” as well for consistency 7. Check the manuscript for some misspelled words (e.g. exsactly), page 9 8. Repetition of the phrase “These values are at par	

	with the results of Singh et al. (2019) at 0.97 for their MaxEnt-based model for the invasion potential of mango fruit borer, Citripestis eutraphera (Lepidoptera: Pyralidae) in India and with Yeh et al. (2021) who used the same modelling framework to assess the potential invasion quarantine pests at 0.84-0.9.” in page 11. please check 9. The reviewer suggests to use Almarinez et al. (2021) instead of Almarinez and colleagues (2021) 10. The reviewer suggests to include the author and year of all the latin names if first mentioned in the text (e.g. Citripestis eutraphera) 11. Some of the listed references are not properly cited in the text (e.g. AGUIRRE-GUTIE´RREZ et al. 2013) and some citation in the text are not listed in the references (e.g. Wan and Yang, 2015 or is it 2016? Guillemaud et al., 2011) please check once again	

	the whole manuscript to properly cite and list all the references used in this study	

4

Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit

(28 Januari 2024)

Thank you.

Respectfully yours,

MJST Editorial Team

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mjstustp, Journal editor, COMMENTS&JUSTIFICATION_ECBE1237.docx

Dear MJST Editorial Team,

Thank you for the information. I will immediately revise the manuscript according to the reviewers' suggestions.

Best Regards,

Yeni Yeni

yenikind
Jan 15

Dear MJST Editorial Team,

In this message, I send the revised manuscript according to the reviewer's suggestions and a comment and justification form.

Best Regards,

Yeni Yeni

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yenikind, Author, Comment and justification form.docx

yenikind
Jan 28

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COMMENTS AND JUSTIFICATION FORM

Paper Code: ECBE1237
Paper Title: In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

First Reviewer:

Comment/s	Justification/s
Those suggestions which would improve the quality of the paper but are not essential for publication.	
1. Overall the manuscript is well-written and well organized.	
2. Technical-sounded	
Changes which should be made before publication.	
1. Should clarify about the problem/motivation that motivates this work and differentiate this work with existing studies.	The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020). Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50

	nM (Yeni & Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra & Colombo, 2023; Kalita & Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020). ……. Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.
2. The references are kind of dated, should be updated with recent reference to keep the work relevant.	References have been updated. Refer to References section.

Second Reviewer:

Comment/s	Justification/s
Those suggestions which would improve the quality of the paper but are not essential for publication.	
1. What was the rationale behind conducting rigid docking in this study? Kindly provide further clarification.	The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).
2. The author fails to provide an explanation of the process of selecting candidate epitopes for docking.	Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on

	antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni & Tjahjono, 2017).
3. Prior to conducting molecular docking and dynamics experiments, the author should first make predictions regarding the immunogenicity and allergenicity of the epitope.	Antigenicity prediction has been carried out on 91 IDH1(R132H) Epitopes Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni & Tjahjono, 2017).
4. The authors exclude any discussion of their research findings and fail to make any comparisons with prior studies.	When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022). The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha &

	Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021).
5. The study lacks a clear delineation of its limitations.	……. Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.
Changes which should be made before publication.	
1. This paper presents the range expansion of Florida wax Scale (FWS) as affected by Local agricultural trade and climate change. The reviewer finds the paper having a profound contribution to the body of knowledge. The reviewer also finds this ecological niche modelling paper very timely and relevant as we are facing the climate change and its inevitable effects. This paper presents and discuss clearly how bioclimatic and anthropocentric factors (i.e.Local Agricultural Trade) could affect the	The comment is unrelated to this manuscript.

distribution and expansion of FWS in the current situation and future situation. This study could also be done for other taxa of economic importance. Further, this could be done as well for species which are forest dependent or are considered bioindicators as they are the species which are greatly affected by the high rate of habitat destruction nowadays. Based on the conclusion provided, this paper could be a basis in any decision making in the future based on the predicted scenario. The reviewer also commends the authors for providing recommendations in order to manage the pests and for providing perspective on what other researchable areas should be explored. Overall the reviewer finds the paper very well written and an excellent addition to the body of knowledge. The reviewer is recommending this paper for publication in the journal. However, the reviewer has very few comments and suggestions for the improvement of the manuscript before publication.	
2. The reviewer has seen a sentence in the introduction where a period is written before the citation. Period should be removed before the citation in the text (.... And outbreak. (Wan and Yang, 2015)). But I think this was just typographical error, but needs to be corrected. Please check whole manuscript for the same typo error.	The comment is unrelated to this manuscript.
3. The authors mentioned at the end part of the introduction that there are the five species of Ceroplastes known to occur in the Philippines. The reviewer suggests that the authors should mention these five species in the text.	The comment is unrelated to this manuscript.
4. The initial taxonomic identification of the wax scales being controversial was reinforced with whole genome sequencing and its identity was confirmed as C. floridensis (DA-BPI-National Plant Quarantine Services Division, personal communication, August 26, 2021)-Accession code should be cited in the paper.	The comment is unrelated to this manuscript.
5. The photo of the FWS in figure 1 is not of the best quality. This could be improved by adding a much clearer photo/s.	The comment is unrelated to this manuscript.

6. Figure 6 should be labelled with "a or b" as well for consistency	The comment is unrelated to this manuscript.
7. Check the manuscript for some misspelled words (e.g. exsactly), page 9	The comment is unrelated to this manuscript.
8. Repetition of the phrase "These values are at par with the results of Singh et al. (2019) at 0.97 for their MaxEnt-based model for the invasion potential of mango fruit borer, Citripestis eutraphera (Lepidoptera: Pyralidae) in India and with Yeh et al. (2021) who used the same modelling framework to assess the potential invasion quarantine pests at 0.84-0.9." in page 11. please check	The comment is unrelated to this manuscript.
9. The reviewer suggests to use Almarinez et al. (2021) instead of Almarinez and colleagues (2021)	The comment is unrelated to this manuscript.
10. The reviewer suggests to include the author and year of all the latin names if first mentioned in the text (e.g. Citripestis eutraphera)	The comment is unrelated to this manuscript.
11. Some of the listed references are not properly cited in the text (e.g. AGUIRRE-GUTIE´RREZ et al. 2013) and some citation in the text are not listed in the references (e.g. Wan and Yang, 2015 or is it 2016? Guillemaud et al., 2011) please check once again the whole manuscript to properly cite and list all the references used in this study	The comment is unrelated to this manuscript.

Third Reviewer:

Comment/s	Justification/s
Changes which should be made before publication.	
1. In the introduction section. last paragraph – "Homology modelling of 91 epitopes of IDH1 (R132H) … for predicting curative activity." For the benefit of readers, here authors need to provide at least some details of rationale behind selecting 91 epitopes, which were previously published in a non-English journal elsewhere (Yeni and Tjahjono, 2017). In the absence of that, it is hard for a reader to learn about selection of 91 peptide sequences. Moreover, all the reader of this journal are not specialized in	The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin

immunology, therefore authors should also explain, about using MHC II and EphA3 receptors for predicting preventative and curative activity, resepctively.	type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020). Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni & Tjahjono, 2017).
2. In abstract "The epitope 42-MHC II complex proved unstable in GROMACS..." is a vague statement. Are they interpreting a conformational transition around 12ns, as a measure of instability? Authors should also need to check the exact cause of large fluctuations at the early stage of simulation in 42-MHC complex (Fig.3). The possible reason could be, either improper minimization, preparation of structural complex, parameter issues etc. It is advisable to redo the simulation.	The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Due to limited facilities and time, it is difficult for the author to repeat the molecular dynamics simulations or add other data.
3. Pl describe in the method section about nature of DOCK grid score. Also explain why none of epitope in Table 2 has significantly lower score than the control A2 peptide. Also it is advisable to evaluate the ranking of protein-peptide complexes using MM-GBSA (or PBSA) Scoring.	An explanation of the grid score can be found in the results and discussion section. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

	Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope 42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy & Stern, 1997; Wang et al., 2022). Due to limited facilities and time, it is difficult for the author to add other data.
4. The language of the manuscript has several issues of improper use of technical words, grammar etc. It should be thoroughly revised. Some of them are mentioned in the following lines.	The choice of words and grammar in the manuscript have been corrected according to the reviewer's suggestions.
5. In abstract and other places in manuscript– "…the greatest preventative and curative activity…" I guess more appropriate usage would be "…high-affinity epitopes as a plausible candidates of preventive and curative activity…". There is a difference between binding affinity and activity.	Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex.
6. In abstract - "The glioma prevention function of the epitopes can be predicted." Authors through computational methods are attempting prediction of binding affinity and stability of receptor-ligand interaction (and not function prediction).	The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7.
7. In the method section, "During the docking process, epitopes and receptors were prepared by adding hydrogen and charge, then surface manufacture using Chimera…" Please redraft it. Manufacturing of what? As a general suggestion, I would also advise authors to use prevalent technical words.	During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2.
8. In Sec 2.2:- Correct the grammar of the following sentence – "Visualisation of molecular dynamics simulations can use VMD…".	Visualization of the molecular dynamics simulations could be carried out using VMD version 1.9.2.

9. In Sec 3.1: "…the stronger the connection between the epitope and the receptor." Replace "Connection" to either "binding" or "interaction."	The more negative the grid score, the stronger the interaction between epitope and the receptor.
10. In introduction - "Some docking study and molecular dynamics simulation devices were utilised …" Replace "devices" to "methods" and also "Some docking study" with "Docking"	Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.
11. Add correct citation of GROMACS as mentioned in https://doi.org/10.5281/zenodo.10017699	Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage.

In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative glioma vaccination were epitope 42 and 54, respectively.

Keywords: Epitope, Glioma, IDH1 (R132H), In Silico, Vaccines

1. Introduction

Brain tumors account for approximately 85-90% of all central nervous system cancers, with glioma being the most prevalent type (Colopi et al., 2023). Furthermore, glioma is a malignant primary brain tumor that originates from glial cells (Delgado-Martín and Medina, 2020). In recent years, scientists have made significant efforts to identify the genetic basis of this condition. This information is expected to help in the development of more effective therapies for patients with a poor prognosis (Choi et al., 2023; Ko and Brody, 2021).

The body relies on the enzyme isocitrate dehydrogenase 1 (IDH1) to produce adenosine triphosphate (ATP) through the citric acid cycle. However, mutations in IDH1 can cause the production of an oncometabolite, namely 2-hydroxyglutarate (Karpel-Massler et al., 2019; Tangella et al., 2023). Although a somatic mutation in codon 132 of the IDH1 gene on locus chromosome 2q33 has been identified in a few glioblastomas cases, it has been found in several low-grade glioma (Ahsan, 2022; Hasanzadeh and Niknejad, 2021; Senhaji et al., 2022; Testa et al., 2020). Among the six different mutations of IDH1, the variation at R132H, in which arginine transforms into histidine is the most frequent (>85%) (Arita et al., 2015; Franceschi et

al., 2021; Matteo et al., 2017; Shayanfar et al., 2023). IDH1 (R132H) can be a biomarker for the presence of glioma (Fujita et al., 2022; Mirchia and Richardson, 2020).

IDH1 (R132H) has been reported to have potential as a tumor-specific neoantigen and is a promising target for immunotherapy. The enzyme contains immunogenic epitopes that are suitable for vaccination (Platten et al., 2021; Yu et al., 2022). Cancer vaccines can be divided into 2 major categories based on their intended usage, namely prophylactic and therapeutic. Prophylactic vaccines are often used to prevent cancer, while therapeutic variants are applied to treat the condition and build body resistance (Kaczmarek et al., 2023; Xinyi Zhang et al., 2023). Furthermore, peptide-based vaccines can be produced by generating antigenic peptides from proteins produced by the tumor cells of interest. It is also important to predict whether the peptides are likely to bind to specific MHC molecules in humans to ensure the efficacy of the therapy developed (Abd-Aziz and Poh, 2022).

The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020).

Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra and Colombo, 2023; Kalita and Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020).

Docking studies have been instrumental in computer-aided drug design (CADD) and are often used for virtual screening or lead optimization in drug screening. Protein-ligand or protein-protein docking studies can be used to predict the direction of a ligand when it is bound to a protein receptor or enzyme (Siebenmorgen and Zacharias, 2020; Supandi et al., 2021; Yeni et al., 2020, 2021). Furthermore, molecular dynamics simulation is a method that is often utilized to comprehend the physical underpinnings of the structure and function of biological macromolecules. During simulation, proteins have a dynamic model, in which internal motions and conformational changes are crucial to their function (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). The root-mean-square deviation (RMSD) graph initially exhibits a steep slope for the first few nanoseconds (ns) and then stabilizes around a constant average value for the rest of the process. The root-mean-square fluctuations (RMSF) graph can be used to illustrate the magnitude of fluctuations of every atom or residue in the protein (Abraham et al., 2023). Based on these findings, this

study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

2. Methodology

2.1 Docking Studies

The docking study was carried out using Dock version 6.7 based on the method proposed in a previous report (Theresa et al., 2015). IDH1 (R132H) epitopes (Table 1) were docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the EphA3 (PDB: 4TWO) receptor, which had been separated with native ligands using Discovery Studio version 16.1.0.15350. Furthermore, the structure of the receptors was obtained from the Protein Data Bank (http://rcsb.org/). The native ligand for MHC II and EphA3 was A2 peptide and compound 164, respectively. The docking method used in this study was rigid body docking, which was proposed by previous studies (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020). Redocking between the receptors and their native ligand was performed before epitopes were docked to obtain RMSD ≤2 Å (Bagheri et al., 2020; Elhady et al., 2021; Ferrari and Patrizio, 2021; Xiangyu Zhang et al., 2021; L. Zheng et al., 2022).

Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccines

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS
20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI

21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2. The spherical form of the samples was then formed to obtain several clusters. Subsequently, one cluster of the receptors with the greatest number of spheres and native ligands was selected for further experimentation. A box was then created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid, and the process was continued with redocking. The redocking grid was also used for docking epitope to the receptors. The grid score was then obtained from the docking results, where a negative value indicated the presence of a greater affinity for epitope-receptor bond. The results were visualized using Discovery Studio, and epitope with the most negative grid score was selected for molecular dynamics simulation.

2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage. Furthermore, the simulation was performed for 25 ns and the LINCS algorithm was used in the AMBER99SB-ILDN force field. The structural changes observed were then analyzed based on the value of RMSD. Visualization of the molecular dynamics simulations could be carried out using VMD version 1.9.2 (Mackoy et al., 2021; Spivak et al., 2023).

3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligands of MHC II and EphA3 were peptide A2 and compound 164, respectively. Furthermore, the results of redocking comprised RMSD value for MHC II with the A2 peptide (0.7371 Å) as well as receptor EphA3 with compound 164 (1.2801 Å). When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). RMSD

values obtained from the process were <2 Å, indicating that the method could be used for virtual screening using epitope.

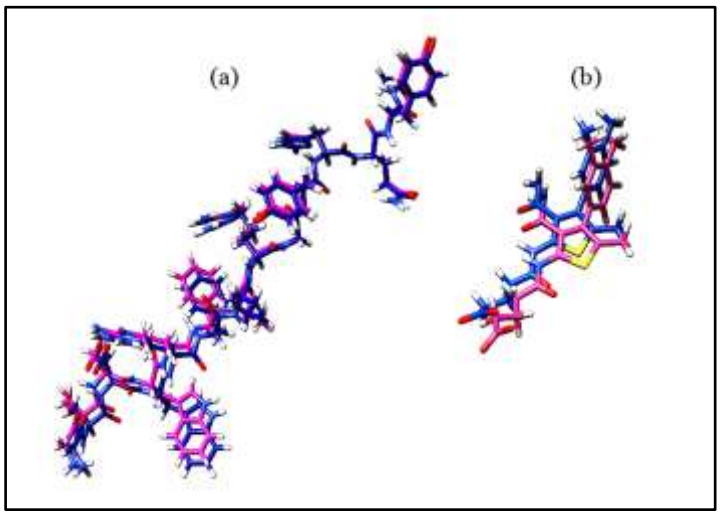

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).

Epitope activity for glioma prevention and treatment was determined based on docking results for MHC II (Table 2) and EphA3 receptor (Table 3). Furthermore, the grid score was obtained from the results. The more negative the grid score, the stronger the interaction between epitope and the receptor. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

Table 2. Results of epitopes docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitopes docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of docking with MHC II obtained the most negative grid score of -62.73 kcal/mol with seven hydrogen bonds from epitope 42, as shown in Figure 2a. Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope

42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy and Stern, 1997; Wang et al., 2022). Meanwhile, the results of docking with the EphA3 receptor showed the most negative grid score of -55.56 kcal/mol with 11 hydrogen bonds on epitope 54, as shown in Figure 2b. This value was more negative compared to the score obtained for redocking compound 164 to the EphA3 receptor.

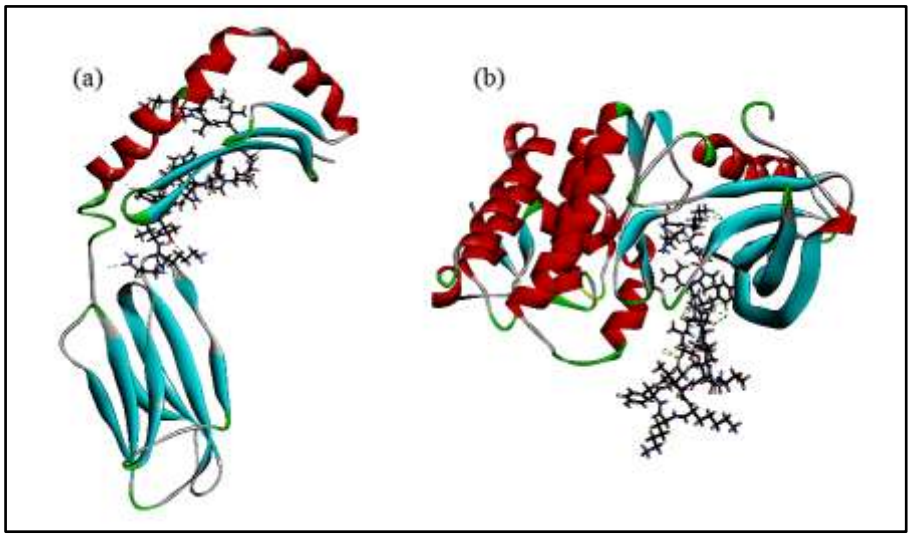

Figure 2. Visualization of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on epitope 42-MHC II and epitope 54-EphA3 receptor complexes. During the process, observation showed that epitope and receptors were flexible (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). Furthermore, the simulations were carried out for 25 ns to determine the stability of docked epitope-receptor complexes.

The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha and Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation.

During the molecular dynamics simulation of epitope 54-EphA3 receptor complex, RMSD fluctuations were stable from the beginning of the process up to 15.29 ns. Subsequently, the value increased drastically to 17 Å and remained stable at 15.48 ns. The results showed that RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å, as shown in Figure 5. At 15 ns in the simulation, the 3D form of epitope 54 changed from a coil to a β-sheet on the last seven amino acids, namely Asn, Thr, Ile, Leu, Lys, Tyr, and Asp, as shown in Figure 6.

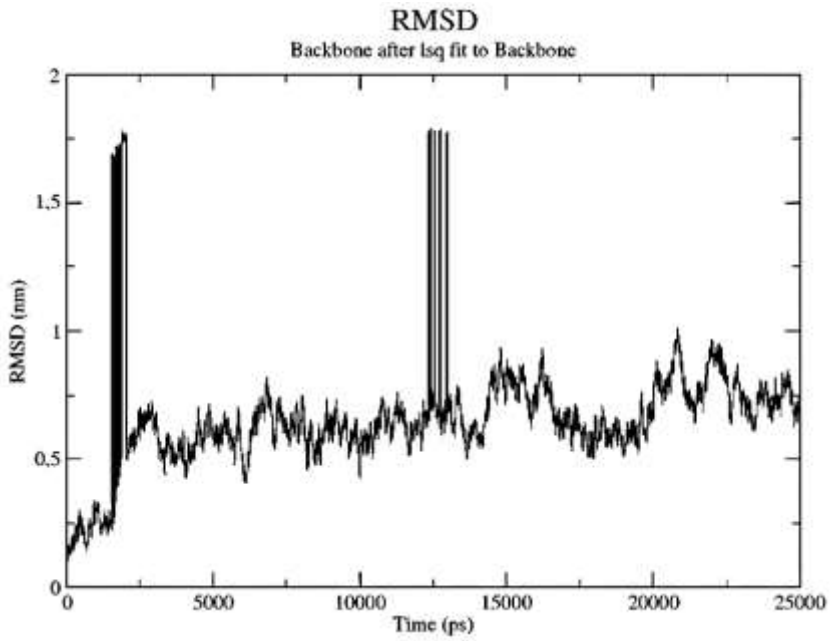

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

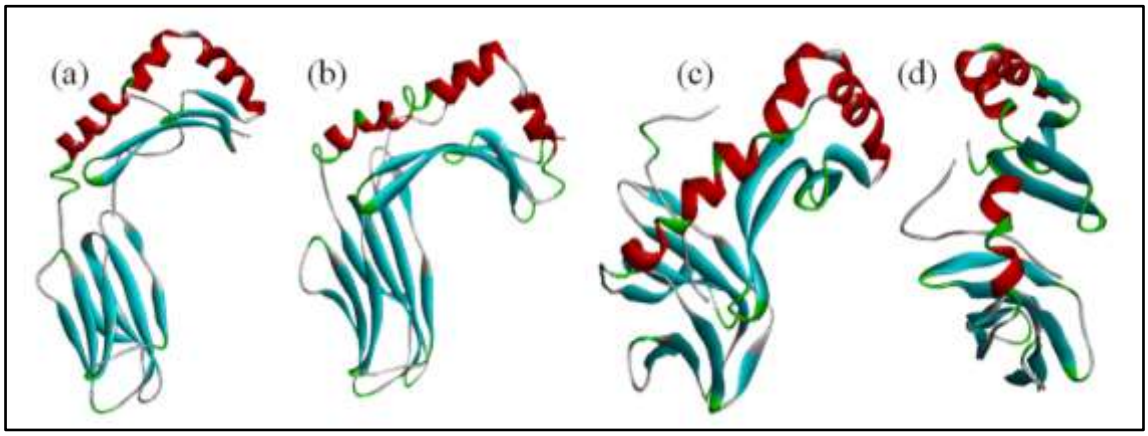

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process, as shown in Figure 7. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021). Based on the results, the number of epitope atoms that fluctuated was higher compared to receptors. Atoms of epitope 42 and 54 began from the 3013th and 4450th atomic orders of the complex, respectively.

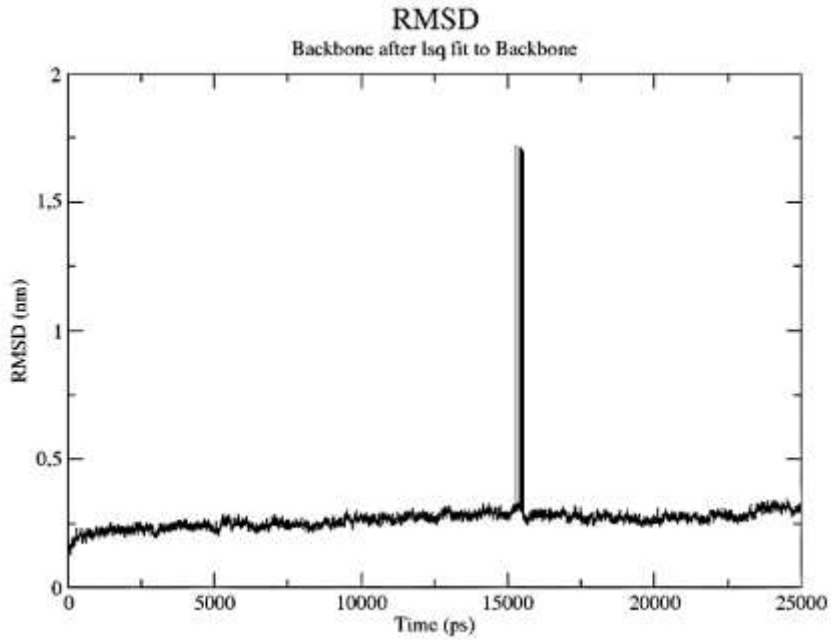

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

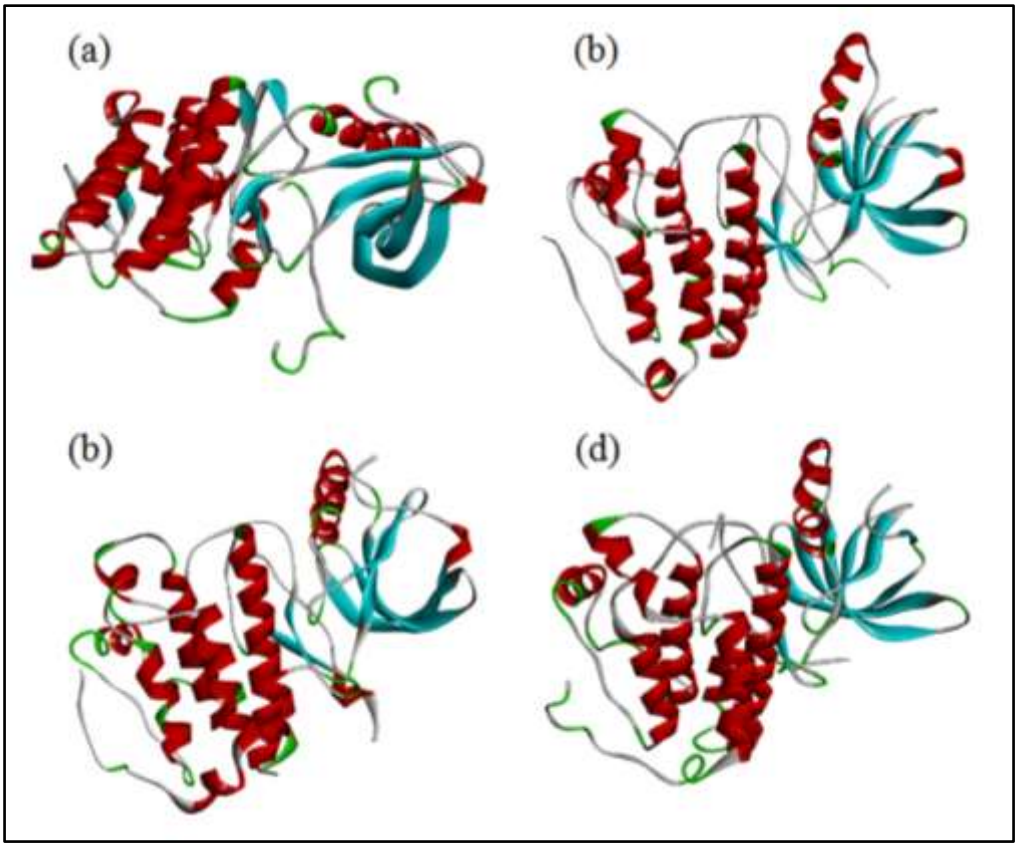

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

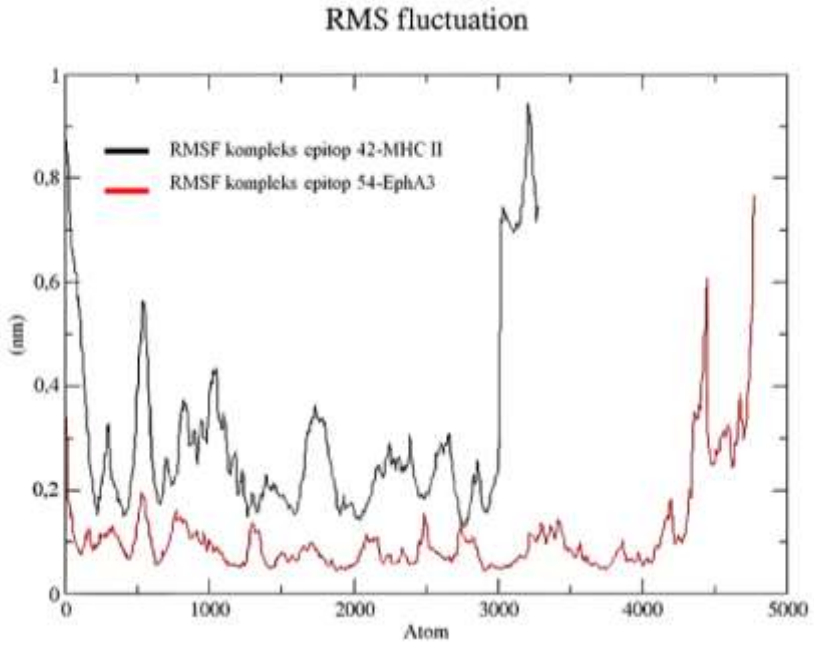

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The best glioma prophylactic and therapeutic vaccines among the 91 epitopes of IDH1 (R132H) were samples 42 and 54, respectively. The grid score of epitope 42 docking into MHC II was -62.73 kcal/mol, while a value of -55.56 kcal/mol was obtained for docking epitope 54 into the EphA3 receptor. During molecular dynamics simulation with a temperature of 300 K, epitope 42-MHC II complex was unstable throughout the process. Meanwhile, the results showed that epitope 54-EphA3 complex was stable from the beginning of the process up to 15.29 ns. Based on these findings, it is important to synthesize epitope 42 and 54 as well as carry out further experimental testing in vitro with the Hs 683 cell line and in vivo to confirm their preventive and curative activity against glioma.

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Bukti konfirmasi review dan hasil review kedua

(13 Februari 2024)

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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative glioma vaccination were epitope 42 and 54, respectively.

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Keywords: Epitope, Glioma, IDH1 (R132H), In Silico, Vaccines

1. Introduction

Brain tumors account for approximately 85-90% of all central nervous system cancers, with glioma being the most prevalent type (Colopi et al., 2023). Furthermore, glioma is a malignant primary brain tumor that originates from glial cells (Delgado-Martín and Medina, 2020). In recent years, scientists have made significant efforts to identify the genetic basis of this condition. This information is expected to help in the development of more effective therapies for patients with a poor prognosis (Choi et al., 2023; Ko and Brody, 2021).

The body relies on the enzyme isocitrate dehydrogenase 1 (IDH1) to produce adenosine triphosphate (ATP) through the citric acid cycle. However, mutations in IDH1 can cause the production of an oncometabolite, namely 2-hydroxyglutarate (Karpel-Massler et al., 2019; Tangella et al., 2023). Although a somatic mutation in codon 132 of the IDH1 gene on locus chromosome 2q33 has been identified in a few glioblastomas cases, it has been found in several low-grade glioma (Ahsan, 2022; Hasanzadeh and Niknejad, 2021; Senhaji et al., 2022; Testa et al., 2020). Among the six different mutations of IDH1, the variation at R132H, in which arginine transforms into histidine is the most frequent (>85%) (Arita et al., 2015; Franceschi et

al., 2021; Matteo et al., 2017; Shayanfar et al., 2023). IDH1 (R132H) can be a biomarker for the presence of glioma (Fujita et al., 2022; Mirchia and Richardson, 2020).

IDH1 (R132H) has been reported to have potential as a tumor-specific neoantigen and is a promising target for immunotherapy. The enzyme contains immunogenic epitopes that are suitable for vaccination (Platten et al., 2021; Yu et al., 2022). Cancer vaccines can be divided into 2 major categories based on their intended usage, namely prophylactic and therapeutic. Prophylactic vaccines are often used to prevent cancer, while therapeutic variants are applied to treat the condition and build body resistance (Kaczmarek et al., 2023; Xinyi Zhang et al., 2023). Furthermore, peptide-based vaccines can be produced by generating antigenic peptides from proteins produced by the tumor cells of interest. It is also important to predict whether the peptides are likely to bind to specific MHC molecules in humans to ensure the efficacy of the therapy developed (Abd-Aziz and Poh, 2022).

The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020).

Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra and Colombo, 2023; Kalita and Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020).

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Docking studies have been instrumental in computer-aided drug design (CADD) and are often used for virtual screening or lead optimization in drug screening. Protein-ligand or protein-protein docking studies can be used to predict the direction of a ligand when it is bound to a protein receptor or enzyme (Siebenmorgen and Zacharias, 2020; Supandi et al., 2021; Yeni et al., 2020, 2021). Furthermore, molecular dynamics simulation is a method that is often utilized to comprehend the physical underpinnings of the structure and function of biological macromolecules. During simulation, proteins have a dynamic model, in which internal motions and conformational changes are crucial to their function (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). The root-mean-square deviation (RMSD) graph initially exhibits a steep slope for the first few nanoseconds (ns) and then stabilizes around a constant average value for the rest of the process. The root-mean-square fluctuations (RMSF) graph can be used to illustrate the magnitude of fluctuations

of every atom or residue in the protein (Abraham et al., 2023). Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

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2. Methodology

2.1 Docking Studies

The docking study was carried out using Dock version 6.7 based on the method proposed in a previous report (Theresa et al., 2015). IDH1 (R132H) epitopes (Table 1) were docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the EphA3 (PDB: 4TWO) receptor, which had been separated with native ligands using Discovery Studio version 16.1.0.15350. Furthermore, the structure of the receptors was obtained from the Protein Data Bank (http://rcsb.org/). The native ligand for MHC II and EphA3 was A2 peptide and compound 164, respectively. The docking method used in this study was rigid body docking, which was proposed by previous studies (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020). Redocking between the receptors and their native ligand was performed before epitopes were docked to obtain RMSD ≤2 Å (Bagheri et al., 2020; Elhady et al., 2021; Ferrari and Patrizio, 2021; Xiangyu Zhang et al., 2021; L. Zheng et al., 2022).

Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccines

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS

20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI
21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2. The spherical form of the samples was then formed to obtain several clusters. Subsequently, one cluster of the receptors with the greatest number of spheres and native ligands was selected for further experimentation. A box was then created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid, and the process was continued with redocking. The redocking grid was also used for docking epitope to the receptors. The grid score was then obtained from the docking results, where a negative value indicated the presence of a greater affinity for epitope-receptor bond. The results were visualized using Discovery Studio, and epitope with the most negative grid score was selected for molecular dynamics simulation.

2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage. Furthermore, the simulation was performed for 25 ns and the LINCS algorithm was used in the AMBER99SB-ILDN force field. The structural changes observed were then analyzed based on the value of RMSD. Visualization of the molecular dynamics simulations could be carried out using VMD version 1.9.2 (Mackoy et al., 2021; Spivak et al., 2023).

3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligands of MHC II and EphA3 were peptide A2 and compound 164, respectively. Furthermore, the results of redocking comprised RMSD value for MHC II with the A2 peptide (0.7371 Å) as well as receptor EphA3 with compound 164 (1.2801 Å). When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). RMSD

values obtained from the process were <2 Å, indicating that the method could be used for virtual screening using epitope.

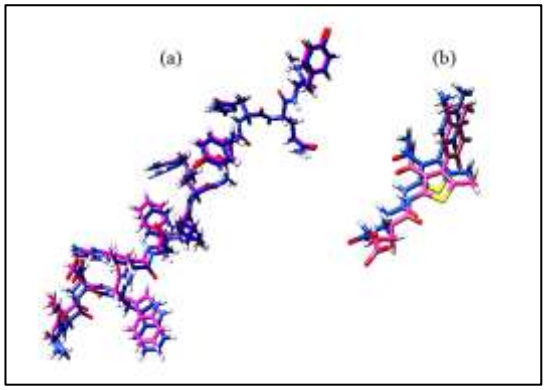

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).

Epitope activity for glioma prevention and treatment was determined based on docking results for MHC II (Table 2) and EphA3 receptor (Table 3). Furthermore, the grid score was obtained from the results. The more negative the grid score, the stronger the interaction between epitope and the receptor. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

Table 2. Results of epitopes docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitopes docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of docking with MHC II obtained the most negative grid score of -62.73 kcal/mol with seven hydrogen bonds from epitope 42, as shown in Figure 2a. Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope 42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy and Stern, 1997; Wang et al., 2022). Meanwhile, the results of docking with the EphA3 receptor showed the most negative grid score of -55.56 kcal/mol with 11 hydrogen bonds on epitope 54, as shown in Figure 2b. This value was more negative compared to the score obtained for redocking compound 164 to the EphA3 receptor.

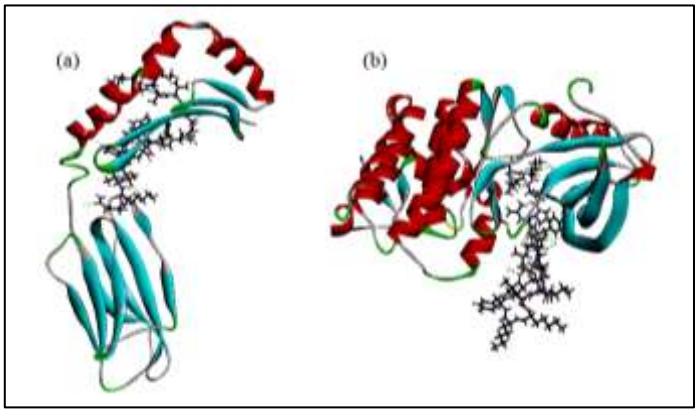

Figure 2. Visualization of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on epitope 42-MHC II and epitope 54-EphA3 receptor complexes. During the process, observation showed that epitope and receptors were flexible (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). Furthermore, the simulations were carried out for 25 ns to determine the stability of docked epitope-receptor complexes.

The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha and Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation.

During the molecular dynamics simulation of epitope 54-EphA3 receptor complex, RMSD fluctuations were stable from the beginning of the process up to 15.29 ns. Subsequently, the value increased drastically to 17 Å and remained stable at 15.48 ns. The results showed that RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å, as shown in Figure 5.

At 15 ns in the simulation, the 3D form of epitope 54 changed from a coil to a β-sheet on the last seven amino acids, namely Asn, Thr, Ile, Leu, Lys, Tyr, and Asp, as shown in Figure 6.

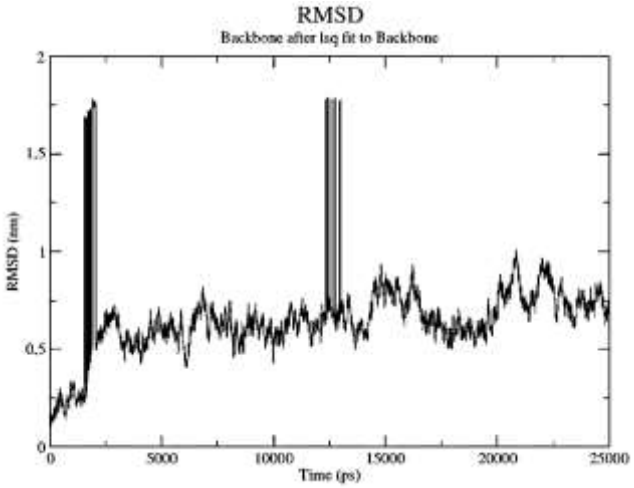

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

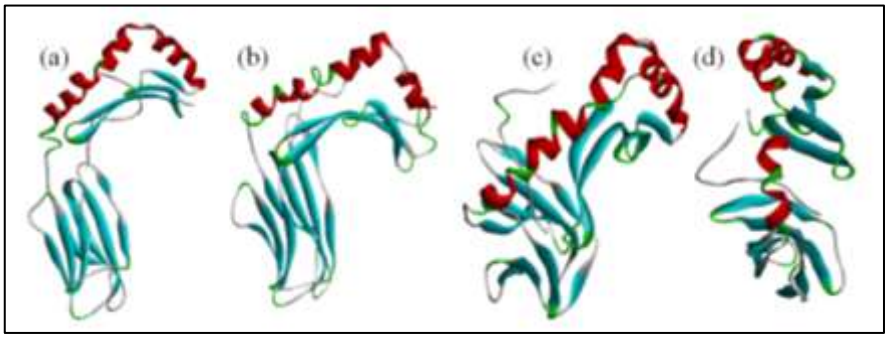

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process, as shown in Figure 7. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021). Based on the

results, the number of epitope atoms that fluctuated was higher compared to receptors. Atoms of epitope 42 and 54 began from the 3013th and 4450th atomic orders of the complex, respectively.

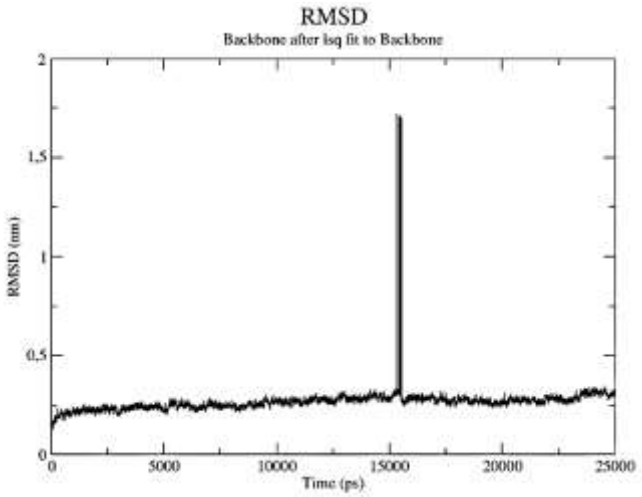

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

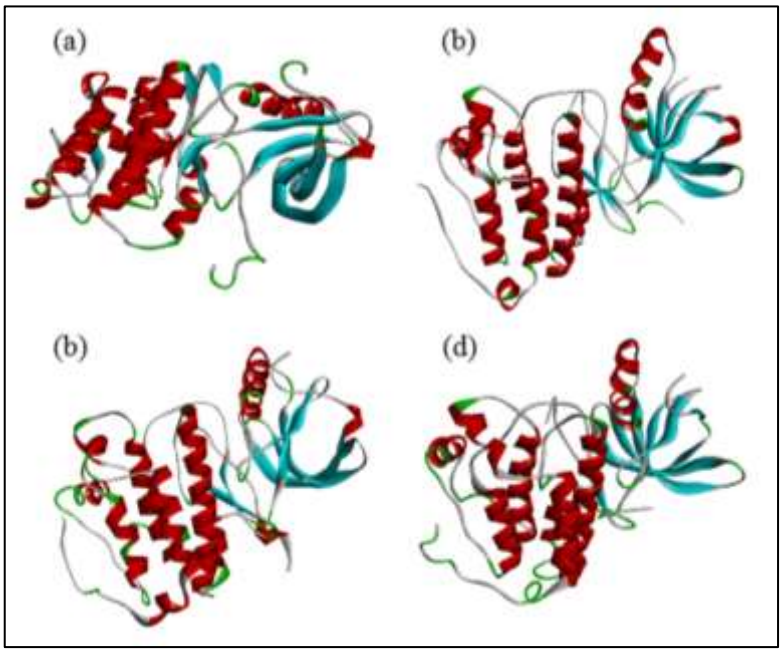

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

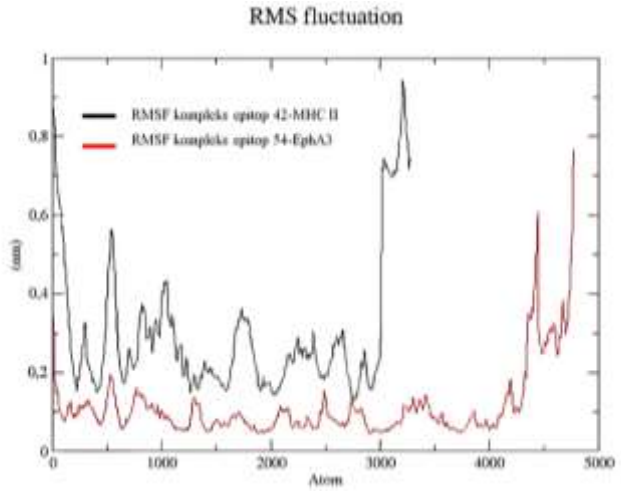

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The best glioma prophylactic and therapeutic vaccines among the 91 epitopes of IDH1 (R132H) were samples 42 and 54, respectively. The grid score of epitope 42 docking into MHC II was -62.73 kcal/mol, while a value of -55.56 kcal/mol was obtained for docking epitope 54 into the EphA3 receptor. During molecular dynamics simulation with a temperature of 300 K, epitope 42-MHC II complex was unstable throughout the process. Meanwhile, the results showed that epitope 54-EphA3 complex was stable from the beginning of the process up to 15.29 ns. Based on these findings, it is important to synthesize epitope 42 and 54 as well as carry out further experimental testing in vitro with the Hs 683 cell line and in vivo to confirm their preventive and curative activity against glioma.

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Paper Code: ECBE1237
Paper Title: In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

Editor:

Comment/s	Justification/s
Change “candidates” to “candidate” and remove “as a” in the following sentence. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation.	Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation.
Add the reference of the following statement. Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures.	Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017).
Change “candidates” to “candidate” and remove “as a” in the following sentence. Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidates of preventive and curative as a glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.	Based on these findings, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative glioma vaccination were epitope 42 and 54, respectively.

Keywords: Epitope, Glioma, IDH1 (R132H), In Silico, Vaccines

1. Introduction

Brain tumors account for approximately 85-90% of all central nervous system cancers, with glioma being the most prevalent type (Colopi et al., 2023). Furthermore, glioma is a malignant primary brain tumor that originates from glial cells (Delgado-Martín and Medina, 2020). In recent years, scientists have made significant efforts to identify the genetic basis of this condition. This information is expected to help in the development of more effective therapies for patients with a poor prognosis (Choi et al., 2023; Ko and Brody, 2021).

The body relies on the enzyme isocitrate dehydrogenase 1 (IDH1) to produce adenosine triphosphate (ATP) through the citric acid cycle. However, mutations in IDH1 can cause the production of an oncometabolite, namely 2-hydroxyglutarate (Karpel-Massler et al., 2019; Tangella et al., 2023). Although a somatic mutation in codon 132 of the IDH1 gene on locus chromosome 2q33 has been identified in a few glioblastomas cases, it has been found in several low-grade glioma (Ahsan, 2022; Hasanzadeh and Niknejad, 2021; Senhaji et al., 2022; Testa et al., 2020). Among the six different mutations of IDH1, the variation at R132H, in which arginine transforms into histidine is the most frequent (>85%) (Arita et al., 2015; Franceschi et

al., 2021; Matteo et al., 2017; Shayanfar et al., 2023). IDH1 (R132H) can be a biomarker for the presence of glioma (Fujita et al., 2022; Mirchia and Richardson, 2020).

IDH1 (R132H) has been reported to have potential as a tumor-specific neoantigen and is a promising target for immunotherapy. The enzyme contains immunogenic epitopes that are suitable for vaccination (Platten et al., 2021; Yu et al., 2022). Cancer vaccines can be divided into 2 major categories based on their intended usage, namely prophylactic and therapeutic. Prophylactic vaccines are often used to prevent cancer, while therapeutic variants are applied to treat the condition and build body resistance (Kaczmarek et al., 2023; Xinyi Zhang et al., 2023). Furthermore, peptide-based vaccines can be produced by generating antigenic peptides from proteins produced by the tumor cells of interest. It is also important to predict whether the peptides are likely to bind to specific MHC molecules in humans to ensure the efficacy of the therapy developed (Abd-Aziz and Poh, 2022).

The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020).

Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of ≥0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra and Colombo, 2023; Kalita and Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020).

Docking studies have been instrumental in computer-aided drug design (CADD) and are often used for virtual screening or lead optimization in drug screening. Protein-ligand or protein-protein docking studies can be used to predict the direction of a ligand when it is bound to a protein receptor or enzyme (Siebenmorgen and Zacharias, 2020; Supandi et al., 2021; Yeni et al., 2020, 2021). Furthermore, molecular dynamics simulation is a method that is often utilized to comprehend the physical underpinnings of the structure and function of biological macromolecules. During simulation, proteins have a dynamic model, in which internal motions and conformational changes are crucial to their function (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). The root-mean-square deviation (RMSD) graph initially exhibits a steep slope for the first few nanoseconds (ns) and then stabilizes around a constant average value for the rest of the process. The root-mean-square fluctuations (RMSF) graph can be used to illustrate the magnitude of fluctuations of every atom or residue in the protein (Abraham et al., 2023). Based on these findings, this

study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

2. Methodology

2.1 Docking Studies

The docking study was carried out using Dock version 6.7 based on the method proposed in a previous report (Theresa et al., 2015). IDH1 (R132H) epitopes (Table 1) were docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the EphA3 (PDB: 4TWO) receptor, which had been separated with native ligands using Discovery Studio version 16.1.0.15350. Furthermore, the structure of the receptors was obtained from the Protein Data Bank (http://rcsb.org/). The native ligand for MHC II and EphA3 was A2 peptide and compound 164, respectively. The docking method used in this study was rigid body docking, which was proposed by previous studies (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020). Redocking between the receptors and their native ligand was performed before epitopes were docked to obtain RMSD ≤2 Å (Bagheri et al., 2020; Elhady et al., 2021; Ferrari and Patrizio, 2021; Xiangyu Zhang et al., 2021; L. Zheng et al., 2022).

Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccines

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS
20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI

21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2. The spherical form of the samples was then formed to obtain several clusters. Subsequently, one cluster of the receptors with the greatest number of spheres and native ligands was selected for further experimentation. A box was then created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid, and the process was continued with redocking. The redocking grid was also used for docking epitope to the receptors. The grid score was then obtained from the docking results, where a negative value indicated the presence of a greater affinity for epitope-receptor bond. The results were visualized using Discovery Studio, and epitope with the most negative grid score was selected for molecular dynamics simulation.

2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage. Furthermore, the simulation was performed for 25 ns and the LINCS algorithm was used in the AMBER99SB-ILDN force field. The structural changes observed were then analyzed based on the value of RMSD. Visualization of the molecular dynamics simulations could be carried out using VMD version 1.9.2 (Mackoy et al., 2021; Spivak et al., 2023).

3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligands of MHC II and EphA3 were peptide A2 and compound 164, respectively. Furthermore, the results of redocking comprised RMSD value for MHC II with the A2 peptide (0.7371 Å) as well as receptor EphA3 with compound 164 (1.2801 Å). When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). RMSD

values obtained from the process were <2 Å, indicating that the method could be used for virtual screening using epitope.

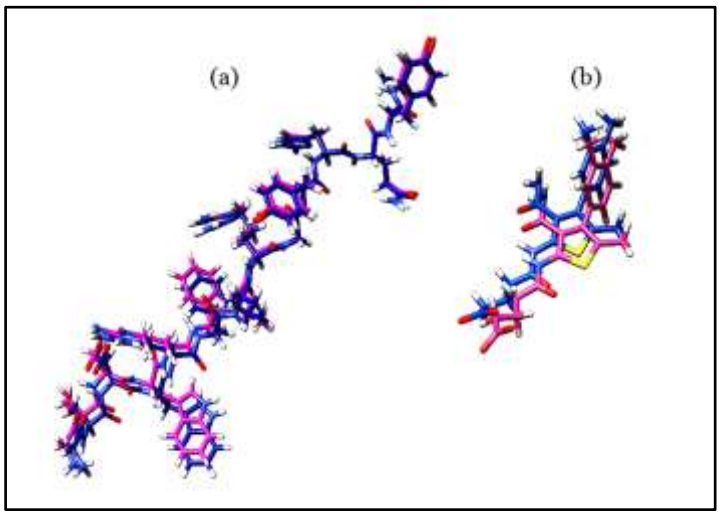

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).

Epitope activity for glioma prevention and treatment was determined based on docking results for MHC II (Table 2) and EphA3 receptor (Table 3). Furthermore, the grid score was obtained from the results. The more negative the grid score, the stronger the interaction between epitope and the receptor. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

Table 2. Results of epitopes docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitopes docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of docking with MHC II obtained the most negative grid score of -62.73 kcal/mol with seven hydrogen bonds from epitope 42, as shown in Figure 2a. Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope

42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy and Stern, 1997; Wang et al., 2022). Meanwhile, the results of docking with the EphA3 receptor showed the most negative grid score of -55.56 kcal/mol with 11 hydrogen bonds on epitope 54, as shown in Figure 2b. This value was more negative compared to the score obtained for redocking compound 164 to the EphA3 receptor.

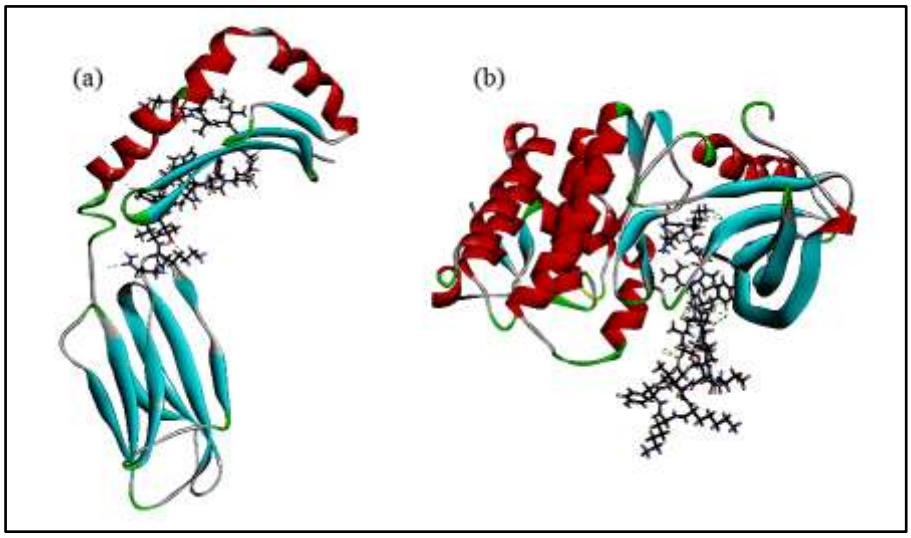

Figure 2. Visualization of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on epitope 42-MHC II and epitope 54-EphA3 receptor complexes. During the process, observation showed that epitope and receptors were flexible (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). Furthermore, the simulations were carried out for 25 ns to determine the stability of docked epitope-receptor complexes.

The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha and Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation.

During the molecular dynamics simulation of epitope 54-EphA3 receptor complex, RMSD fluctuations were stable from the beginning of the process up to 15.29 ns. Subsequently, the value increased drastically to 17 Å and remained stable at 15.48 ns. The results showed that RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å, as shown in Figure 5. At 15 ns in the simulation, the 3D form of epitope 54 changed from a coil to a β-sheet on the last seven amino acids, namely Asn, Thr, Ile, Leu, Lys, Tyr, and Asp, as shown in Figure 6.

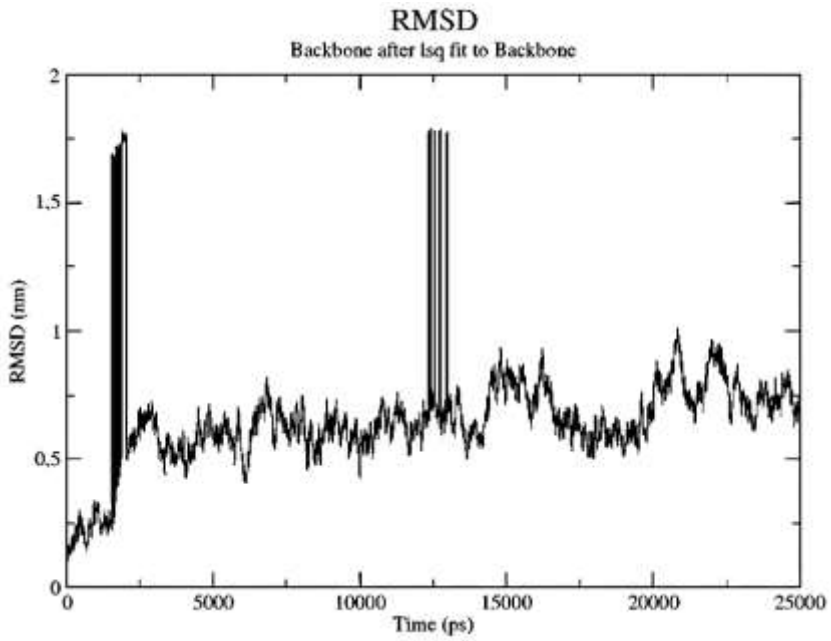

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

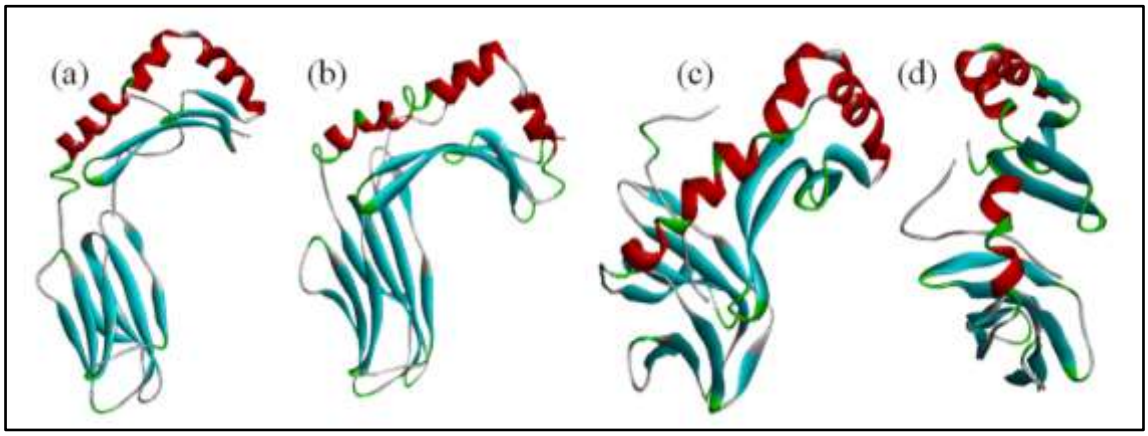

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process, as shown in Figure 7. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021). Based on the results, the number of epitope atoms that fluctuated was higher compared to receptors. Atoms of epitope 42 and 54 began from the 3013th and 4450th atomic orders of the complex, respectively.

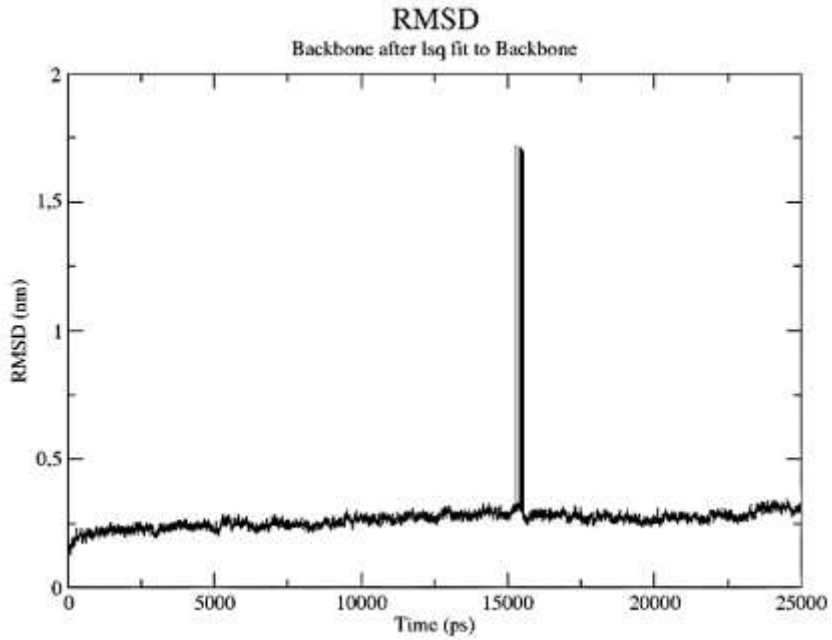

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

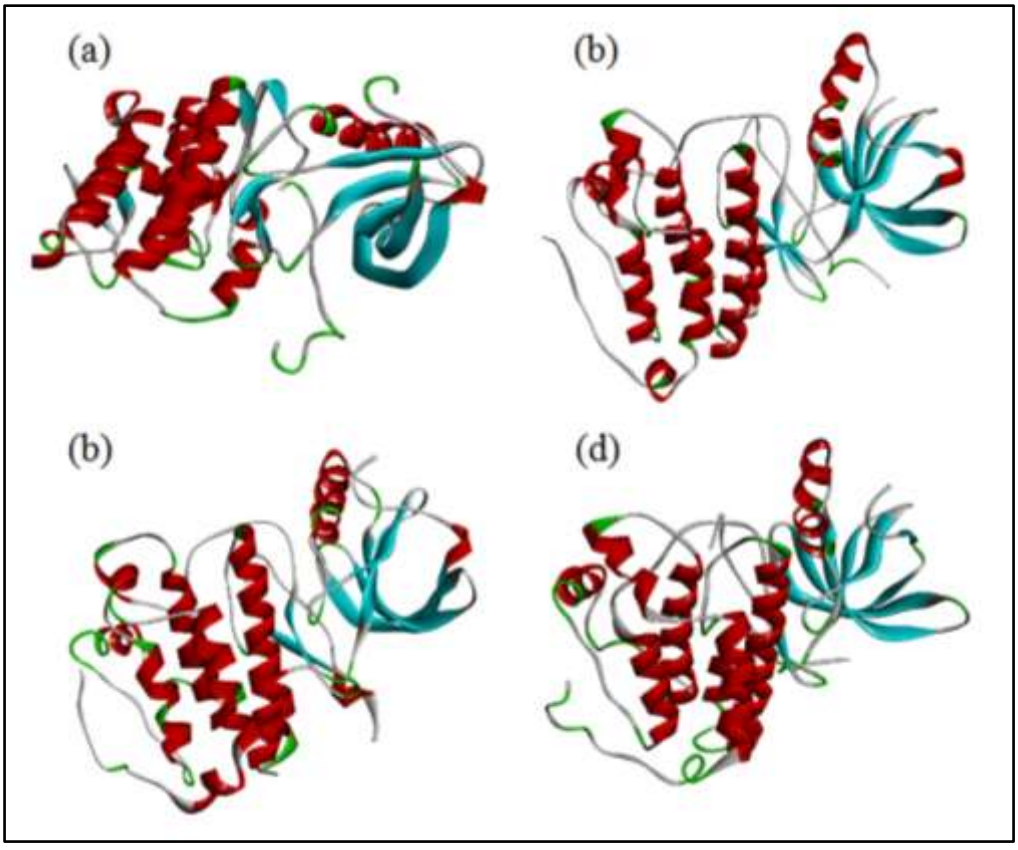

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

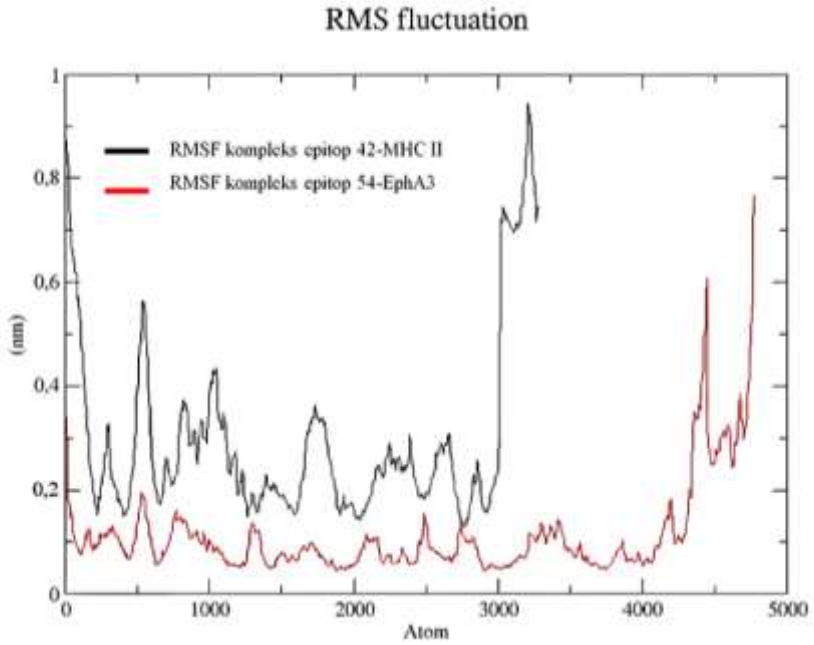

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The best glioma prophylactic and therapeutic vaccines among the 91 epitopes of IDH1 (R132H) were samples 42 and 54, respectively. The grid score of epitope 42 docking into MHC II was -62.73 kcal/mol, while a value of -55.56 kcal/mol was obtained for docking epitope 54 into the EphA3 receptor. During molecular dynamics simulation with a temperature of 300 K, epitope 42-MHC II complex was unstable throughout the process. Meanwhile, the results showed that epitope 54-EphA3 complex was stable from the beginning of the process up to 15.29 ns. Based on these findings, it is important to synthesize epitope 42 and 54 as well as carry out further experimental testing in vitro with the Hs 683 cell line and in vivo to confirm their preventive and curative activity against glioma.

5. References

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Bukti konfirmasi review dan hasil review ketiga
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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative glioma vaccination were epitope 42 and 54, respectively.

Keywords: Epitope, Glioma, IDH1 (R132H), In Silico, Vaccines

1. Introduction

Brain tumors account for approximately 85-90% of all central nervous system cancers, with glioma being the most prevalent type (Colopi et al., 2023). Furthermore, glioma is a malignant primary brain tumor that originates from glial cells (Delgado-Martín and Medina, 2020). In recent years, scientists have made significant efforts to identify the genetic basis of this condition. This information is expected to help in the development of more effective therapies for patients with a poor prognosis (Choi et al., 2023; Ko and Brody, 2021).

The body relies on the enzyme isocitrate dehydrogenase 1 (IDH1) to produce adenosine triphosphate (ATP) through the citric acid cycle. However, mutations in IDH1 can cause the production of an oncometabolite, namely 2-hydroxyglutarate (Karpel-Massler et al., 2019; Tangella et al., 2023). Although a somatic mutation in codon 132 of the IDH1 gene on locus chromosome 2q33 has been identified in a few glioblastomas cases, it has been found in several low-grade glioma (Ahsan, 2022; Hasanzadeh and Niknejad, 2021; Senhaji et al., 2022; Testa et al., 2020). Among the six different mutations of IDH1, the variation at R132H, in which arginine transforms into histidine is the most frequent (>85%) (Arita et al., 2015; Franceschi et

al., 2021; Matteo et al., 2017; Shayanfar et al., 2023). IDH1 (R132H) can be a biomarker for the presence of glioma (Fujita et al., 2022; Mirchia and Richardson, 2020).

IDH1 (R132H) has been reported to have potential as a tumor-specific neoantigen and is a promising target for immunotherapy. The enzyme contains immunogenic epitopes that are suitable for vaccination (Platten et al., 2021; Yu et al., 2022). Cancer vaccines can be divided into 2 major categories based on their intended usage, namely prophylactic and therapeutic. Prophylactic vaccines are often used to prevent cancer, while therapeutic variants are applied to treat the condition and build body resistance (Kaczmarek et al., 2023; Xinyi Zhang et al., 2023). Furthermore, peptide-based vaccines can be produced by generating antigenic peptides from proteins produced by the tumor cells of interest. It is also important to predict whether the peptides are likely to bind to specific MHC molecules in humans to ensure the efficacy of the therapy developed (Abd-Aziz and Poh, 2022).

The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020).

Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of $\geq$0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra and Colombo, 2023; Kalita and Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020).

Docking studies have been instrumental in computer-aided drug design (CADD) and are often used for virtual screening or lead optimization in drug screening. Protein-ligand or protein-protein docking studies can be used to predict the direction of a ligand when it is bound to a protein receptor or enzyme (Siebenmorgen and Zacharias, 2020; Supandi et al., 2021; Yeni et al., 2020, 2021). Furthermore, molecular dynamics simulation is a method that is often utilized to comprehend the physical underpinnings of the structure and function of biological macromolecules. During simulation, proteins have a dynamic model, in which internal motions and conformational changes are crucial to their function (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). The root-mean-square deviation (RMSD) graph initially exhibits a steep slope for the first few nanoseconds (ns) and then stabilizes around a constant average value for the rest of the process. The root-mean-square fluctuations (RMSF) graph can be used to illustrate the magnitude of fluctuations of every atom or residue in the protein (Abraham et al., 2023). Based on these findings, this

study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

2. Methodology

2.1 Docking Studies

The docking study was carried out using Dock version 6.7 based on the method proposed in a previous report (Theresa et al., 2015). IDH1 (R132H) epitopes (Table 1) were docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the EphA3 (PDB: 4TWO) receptor, which had been separated with native ligands using Discovery Studio version 16.1.0.15350. Furthermore, the structure of the receptors was obtained from the Protein Data Bank (http://rcsb.org/). The native ligand for MHC II and EphA3 was A2 peptide and compound 164, respectively. The docking method used in this study was rigid body docking, which was proposed by previous studies (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020). Redocking between the receptors and their native ligand was performed before epitopes were docked to obtain RMSD ≤2 Å (Bagheri et al., 2020; Elhady et al., 2021; Ferrari and Patrizio, 2021; Xiangyu Zhang et al., 2021; L. Zheng et al., 2022).

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Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccines

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS
20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI

21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2. The spherical form of the samples was then formed to obtain several clusters. Subsequently, one cluster of the receptors with the greatest number of spheres and native ligands was selected for further experimentation. A box was then created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid, and the process was continued with redocking. The redocking grid was also used for docking epitope to the receptors. The grid score was then obtained from the docking results, where a negative value indicated the presence of a greater affinity for epitope-receptor bond. The results were visualized using Discovery Studio, and epitope with the most negative grid score was selected for molecular dynamics simulation.

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2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage. Furthermore, the simulation was performed for 25 ns and the LINCS algorithm was used in the AMBER99SB-ILDN force field. The structural changes observed were then analyzed based on the value of RMSD. Visualization of the molecular dynamics simulations could be carried out using VMD version 1.9.2 (Mackoy et al., 2021; Spivak et al., 2023).

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3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligands of MHC II and EphA3 were peptide A2 and compound 164, respectively. Furthermore, the results of redocking comprised RMSD value for MHC II with the A2 peptide (0.7371 Å) as well as receptor EphA3 with compound 164 (1.2801 Å). When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). RMSD

values obtained from the process were <2 Å, indicating that the method could be used for virtual screening using epitope.

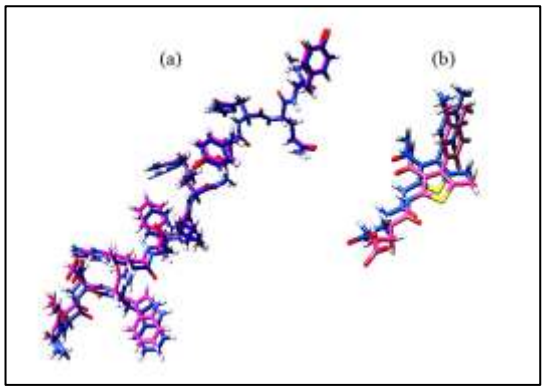

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).

Epitope activity for glioma prevention and treatment was determined based on docking results for MHC II (Table 2) and EphA3 receptor (Table 3). Furthermore, the grid score was obtained from the results. The more negative the grid score, the stronger the interaction between epitope and the receptor. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

Table 2. Results of epitopes docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitopes docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of docking with MHC II obtained the most negative grid score of -62.73 kcal/mol with seven hydrogen bonds from epitope 42, as shown in Figure 2a. Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope

42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy and Stern, 1997; Wang et al., 2022). Meanwhile, the results of docking with the EphA3 receptor showed the most negative grid score of -55.56 kcal/mol with 11 hydrogen bonds on epitope 54, as shown in Figure 2b. This value was more negative compared to the score obtained for redocking compound 164 to the EphA3 receptor.

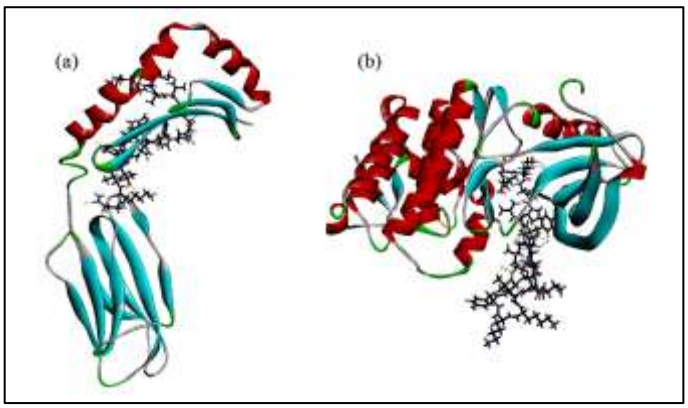

Figure 2. Visualization of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on epitope 42-MHC II and epitope 54-EphA3 receptor complexes. During the process, observation showed that epitope and receptors were flexible (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). Furthermore, the simulations were carried out for 25 ns to determine the stability of docked epitope-receptor complexes.

The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha and Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation.

During the molecular dynamics simulation of epitope 54-EphA3 receptor complex, RMSD fluctuations were stable from the beginning of the process up to 15.29 ns. Subsequently, the value increased drastically to 17 Å and remained stable at 15.48 ns. The results showed that RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å, as shown in Figure 5. At 15 ns in the simulation, the 3D form of epitope 54 changed from a coil to a β-sheet on the last seven amino acids, namely Asn, Thr, Ile, Leu, Lys, Tyr, and Asp, as shown in Figure 6.

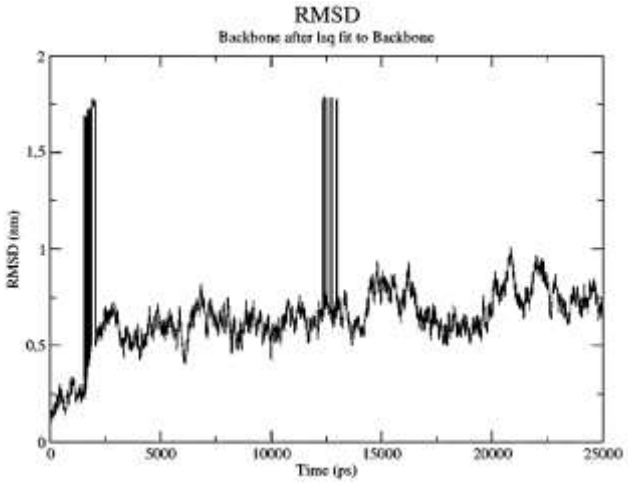

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

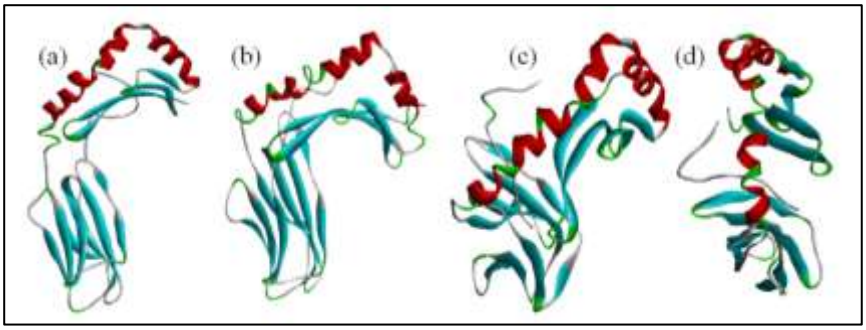

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process, as shown in Figure 7. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021). Based on the results, the number of epitope atoms that fluctuated was higher compared to receptors. Atoms of epitope 42 and 54 began from the 3013th and 4450th atomic orders of the complex, respectively.

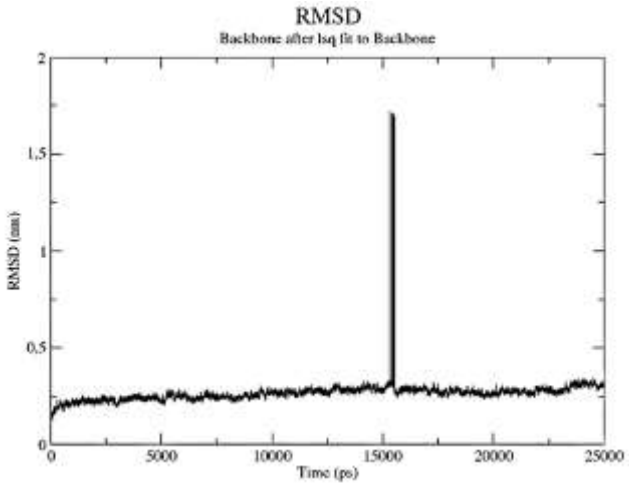

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

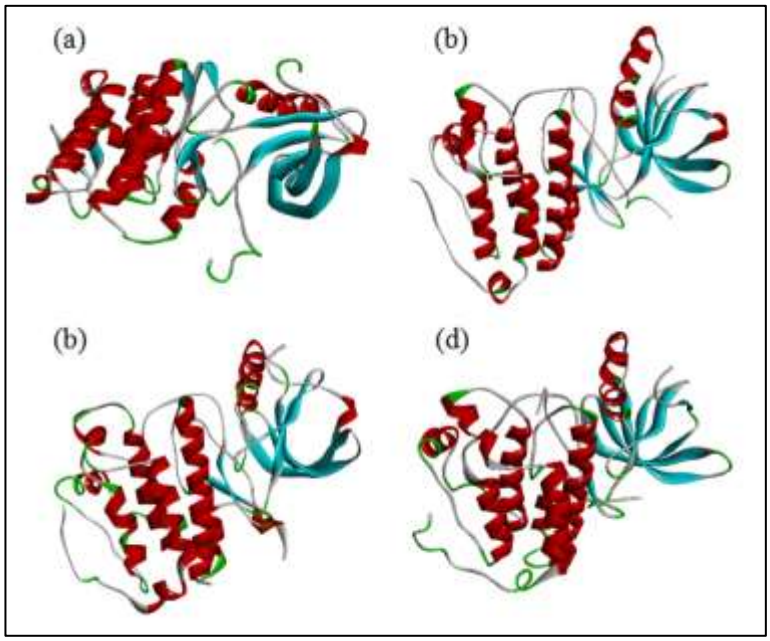

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

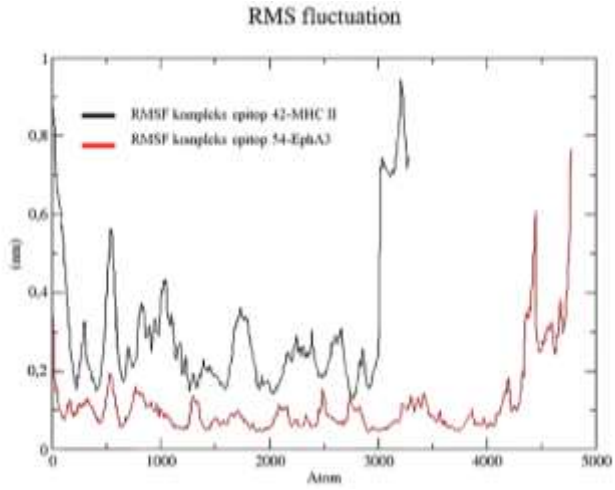

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The best glioma prophylactic and therapeutic vaccines among the 91 epitopes of IDH1 (R132H) were samples 42 and 54, respectively. The grid score of epitope 42 docking into MHC II was -62.73 kcal/mol, while a value of -55.56 kcal/mol was obtained for docking epitope 54 into the EphA3 receptor. During molecular dynamics simulation with a temperature of 300 K, epitope 42-MHC II complex was unstable throughout the process. Meanwhile, the results showed that epitope 54-EphA3 complex was stable from the beginning of the process up to 15.29 ns. Based on these findings, it is important to synthesize epitope 42 and 54 as well as carry out further experimental testing in vitro with the Hs 683 cell line and in vivo to confirm their preventive and curative activity against glioma.

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Yeni Uhamka Klender <yeni@uhamka.ac.id>

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Kindly cite this software (Discovery Studio) properly, and list this in the Reference section. The results were visualized using Discovery Studio, and epitope with the	The results were visualized using Discovery Studio (Jejurikar & Rohane, 2021), and epitope with the most negative grid score was selected for molecular dynamics simulation. Jejurikar, B. L., & Rohane, S. H. (2021). Drug Designing in Discovery Studio. Asian Journal of Research in Chemistry, 14(2), 135-138. https://doi.org/10.5958/0974-4150.2021.

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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD >3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative glioma vaccination were epitope 42 and 54, respectively.

Keywords: Epitope, Glioma, IDH1 (R132H), In Silico, Vaccines

1. Introduction

Brain tumors account for approximately 85-90% of all central nervous system cancers, with glioma being the most prevalent type (Colopi et al., 2023). Furthermore, glioma is a malignant primary brain tumor that originates from glial cells (Delgado-Martín and Medina, 2020). In recent years, scientists have made significant efforts to identify the genetic basis of this condition. This information is expected to help in the development of more effective therapies for patients with a poor prognosis (Choi et al., 2023; Ko and Brody, 2021).

The body relies on the enzyme isocitrate dehydrogenase 1 (IDH1) to produce adenosine triphosphate (ATP) through the citric acid cycle. However, mutations in IDH1 can cause the production of an oncometabolite, namely 2-hydroxyglutarate (Karpel-Massler et al., 2019; Tangella et al., 2023). Although a somatic mutation in codon 132 of the IDH1 gene on locus chromosome 2q33 has been identified in a few glioblastomas cases, it has been found in several low-grade glioma (Ahsan, 2022; Hasanzadeh and Niknejad, 2021; Senhaji et al., 2022; Testa et al., 2020). Among the six different mutations of IDH1, the variation at R132H, in which arginine transforms into histidine is the most frequent (>85%) (Arita et al., 2015; Franceschi et

al., 2021; Matteo et al., 2017; Shayanfar et al., 2023). IDH1 (R132H) can be a biomarker for the presence of glioma (Fujita et al., 2022; Mirchia and Richardson, 2020).

IDH1 (R132H) has been reported to have potential as a tumor-specific neoantigen and is a promising target for immunotherapy. The enzyme contains immunogenic epitopes that are suitable for vaccination (Platten et al., 2021; Yu et al., 2022). Cancer vaccines can be divided into 2 major categories based on their intended usage, namely prophylactic and therapeutic. Prophylactic vaccines are often used to prevent cancer, while therapeutic variants are applied to treat the condition and build body resistance (Kaczmarek et al., 2023; Xinyi Zhang et al., 2023). Furthermore, peptide-based vaccines can be produced by generating antigenic peptides from proteins produced by the tumor cells of interest. It is also important to predict whether the peptides are likely to bind to specific MHC molecules in humans to ensure the efficacy of the therapy developed (Abd-Aziz and Poh, 2022).

The epitopes of IDH1 (R132H) are present in major histocompatibility complexes class II (MHC II) and stimulate mutation-specific $CD4^+$ T helper-1 (TH1) cells. In glioma patients with R132H mutations, $CD4^+$ T helper-1 (TH1) and spontaneous antibodies recognize IDH1 (R132H) preferentially (Bunse et al., 2022). Since all tumor cell surfaces exhibit the enzyme in its R132H form, vaccines can alert the immune system of humans to its presence without causing harm to the healthy cells (Kaczmarek et al., 2023; Liu et al., 2023). Large quantities of the ephrin type-A receptor 3 (EphA3) are expressed in gliomas and mesenchymal subtypes of glioblastoma. EphA3 expression is considerably higher during the early stages of tumor development, where cell differentiation has not yet occurred. EphA3 actively contributes to the maintenance of undifferentiated tumor cells. Furthermore, therapeutic targeting of the EphA3 receptor could be applicable to these tumors (Arora et al., 2023; Baumgartner et al., 2021; J. Zheng et al., 2020).

Homology modeling has been carried out to analyze 91 epitopes of IDH1 (R132H) that show potential as cancer antigens based on antigenicity prediction result with a threshold limit of $\geq$0.4 using VaxiJen, followed by validation and refinement of the structures. These epitopes have been predicted to bind strongly to MHC II allele HLA-DRB10101 because they have an IC_{50} value of less than 50 nM (Yeni and Tjahjono, 2017). Several studies have explored the use of computational methods in identifying compounds. The use of in silico-based methods to predict epitopes for producing peptide vaccines designed rationally has been reported to improve the efficacy of vaccination (Guarra and Colombo, 2023; Kalita and Tripathi, 2022; Rawal et al., 2021; Soleymani et al., 2022; Sunita et al., 2020).

Docking studies have been instrumental in computer-aided drug design (CADD) and are often used for virtual screening or lead optimization in drug screening. Protein-ligand or protein-protein docking studies can be used to predict the direction of a ligand when it is bound to a protein receptor or enzyme (Siebenmorgen and Zacharias, 2020; Supandi et al., 2021; Yeni et al., 2020, 2021). Furthermore, molecular dynamics simulation is a method that is often utilized to comprehend the physical underpinnings of the structure and function of biological macromolecules. During simulation, proteins have a dynamic model, in which internal motions and conformational changes are crucial to their function (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). The root-mean-square deviation (RMSD) graph initially exhibits a steep slope for the first few nanoseconds (ns) and then stabilizes around a constant average value for the rest of the process. The root-mean-square fluctuations (RMSF) graph can be used to illustrate the magnitude of fluctuations of every atom or residue in the protein (Abraham et al., 2023). Based on these findings, this

study aims to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex. Docking and molecular dynamics simulation methods were utilized to determine the affinity of samples against MHC II and EphA3 receptors to predict the preventative and curative activities, respectively.

2. Methodology

2.1 Docking Studies

The docking study was carried out using Dock version 6.7 (Theresa et al., 2015) based on the method proposed in a previous report. IDH1 (R132H) epitopes (Table 1) were docked with MHC II HLA DRB1 0101 (PDB: 1AQD) and the EphA3 (PDB: 4TWO) receptor, which had been separated with native ligands using Discovery Studio version 16.1.0.15350. Furthermore, the structure of the receptors was obtained from the Protein Data Bank (Burley et al., 2022). The native ligand for MHC II and EphA3 was A2 peptide and compound 164, respectively. The docking method used in this study was rigid body docking, which was proposed by previous studies (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020). Redocking between the receptors and their native ligand was performed before epitopes were docked to obtain RMSD ≤2 Å (Bagheri et al., 2020; Elhady et al., 2021; Ferrari and Patrizio, 2021; Xiangyu Zhang et al., 2021; L. Zheng et al., 2022).

Table 1. The amino acid sequence of IDH1 (R132H) epitopes as a candidate glioma vaccines

Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence	Epitope	Amino Acid Sequence
1	QYRATDFVVPGPGKV	32	LAFFANALEEVSIET	63	LVCPDGKTVEAEAAHGTVTR
2	YRATDFVVPGPGKVE	33	DLAACIKGLPNVQRS	64	VCPDGKTVEAEAAHGTVTRH
3	LVHNFEEGGGVAMGM	34	LAACIKGLPNVQRSD	65	CPDGKTVEAEAAHGTVTRHY
4	HNFEEGGGVAMGMYN	35	AACIKGLPNVQRSDY	66	PDGKTVEAEAAHGTVTRHYR
5	SIEDFAHSSFQMALS	36	ACIKGLPNVQRSDYL	67	YQKGQETSTNPIASIFAWTR
6	SSFQMALSKGWPLYL	37	TFEFMDKLGENLKIK	68	QKGQETSTNPIASIFAWTRG
7	SFQMALSKGWPLYLS	38	FEFMDKLGENLKIKL	69	KGQETSTNPIASIFAWTRGL
8	MALSKGWPLYLSTKN	39	KLGENLKIKLAQAKL	70	QETSTNPIASIFAWTRGLAH
9	LSKGWPLYLSTKNTI	40	SKKISGGSVVEMQGDEMTRI	71	ETSTNPIASIFAWTRGLAHR
10	KGWPLYLSTKNTILK	41	KKISGGSVVEMQGDEMTRII	72	TSTNPIASIFAWTRGLAHRA
11	GWPLYLSTKNTILKK	42	QKVTYLVHNFEEGGGVAMGM	73	STNPIASIFAWTRGLAHRAK
12	WPLYLSTKNTILKKY	43	KVTYLVHNFEEGGGVAMGMY	74	TNPIASIFAWTRGLAHRAKL
13	PLYLSTKNTILKKYD	44	TYLVHNFEEGGGVAMGMYNQ	75	PIASIFAWTRGLAHRAKLDN
14	LYLSTKNTILKKYDG	45	VTYLVHNFEEGGGVAMGMYN	76	IFAWTRGLAHRAKLDNNKEL
15	YLSTKNTILKKYDGR	46	SIEDFAHSSFQMALSKGWPL	77	FAWTRGLAHRAKLDNNKELA
16	HRLIDDMVAQAMKSE	47	FAHSSFQMALSKGWPLYLST	78	AWTRGLAHRAKLDNNKELAF
17	RLIDDMVAQAMKSEG	48	AHSSFQMALSKGWPLYLSTK	79	WTRGLAHRAKLDNNKELAFF
18	LIDDMVAQAMKSEGG	49	HSSFQMALSKGWPLYLSTKN	80	RAKLDNNKELAFFANALEEV
19	PDGKTVEAEAAHGTV	50	SSFQMALSKGWPLYLSTKNT	81	AKLDNNKELAFFANALEEVS
20	DGKTVEAEAAHGTVT	51	SFQMALSKGWPLYLSTKNTI	82	KLDNNKELAFFANALEEVSI

21	GKTVEAEAAHGTVTR	52	QMALSKGWPLYLSTKNTILK	83	LDNNKELAFFANALEEVSIE
22	KTVEAEAAHGTVTRH	53	MALSKGWPLYLSTKNTILKK	84	DNNKELAFFANALEEVSIET
23	ASIFAWTRGLAHRAK	54	LSKGWPLYLSTKNTILKKYD	85	FMTKDLAACIKGLPNVQRSD
24	SIFAWTRGLAHRAKL	55	SKGWPLYLSTKNTILKKYDG	86	MTKDLAACIKGLPNVQRSDY
25	FAWTRGLAHRAKLDN	56	KGWPLYLSTKNTILKKYDGR	87	TKDLAACIKGLPNVQRSDYL
26	LDNNKELAFFANALE	57	GWPLYLSTKNTILKKYDGRF	88	SDYLNTFEFMDKLGENLKIK
27	DNNKELAFFANALEE	58	WPLYLSTKNTILKKYDGRFK	89	DYLNTFEFMDKLGENLKIKL
28	NNKELAFFANALEEV	59	PLYLSTKNTILKKYDGRFKD	90	YLNTFEFMDKLGENLKIKLA
29	NKELAFFANALEEVS	60	LYLSTKNTILKKYDGRFKDI	91	FEFMDKLGENLKIKLAQAKL
30	KELAFFANALEEVSI	61	YLSTKNTILKKYDGRFKDIF		
31	ELAFFANALEEVSIE	62	VLVCPDGKTVEAEAAHGTVT		

During the docking process, epitope and receptors were prepared by adding hydrogen and charge, followed by generating surface of receptors using Chimera version 1.10.2 (Huang et al., 2014). The spherical form of the samples was then formed to obtain several clusters. Subsequently, one cluster of the receptors with the greatest number of spheres and native ligands was selected for further experimentation. A box was then created around the active side of the receptor, with an extra margin of 20 Å to be used for making the grid, and the process was continued with redocking. The redocking grid was also used for docking epitope to the receptors. The grid score was then obtained from the docking results, where a negative value indicated the presence of a greater affinity for epitope-receptor bond. The results were visualized using Discovery Studio (Jejurikar & Rohane, 2021), and epitope with the most negative grid score was selected for molecular dynamics simulation.

2.2 Molecular Dynamics Simulations

Molecular dynamics simulations were performed using GROMACS version 5.0.6 (Abraham et al., 2015, 2023), with a temperature of 300 K for epitope-MHC II and epitope-EphA3 complexes, which were selected in the previous stage. Furthermore, the simulation was performed for 25 ns and the LINear Constraint Solver (LINCS) algorithm was used in the AMBER99SB-ILDN force field. The structural changes observed were then analyzed based on the value of the root-mean-square deviation (RMSD). Visualization of the molecular dynamics simulations could be carried out using Visual Molecular Dynamics (VMD) version 1.9.2 (Mackoy et al., 2021; Spivak et al., 2023).

3. Results and Discussion

3.1 Docking Studies

Redocking between receptors with native ligands in the Protein Data Bank (Figure 1) was performed first to validate the docking method. The native ligands of MHC II and EphA3 were peptide A2 and compound 164, respectively. Furthermore, the results of redocking comprised RMSD value for MHC II with the A2 peptide (0.7371 Å) as well as receptor EphA3 with compound 164 (1.2801 Å). When the RMSD value is less than 2 Å, the algorithms and utilized parameters were adequate for determining the optimal docking pose. Therefore, the results obtained from the directed docking protocol are considered valid, ensuring the biological relevance of the docking poses and their corresponding energies (Elhady et al., 2021). RMSD

values obtained from the process were <2 Å, indicating that the method could be used for virtual screening using epitope.

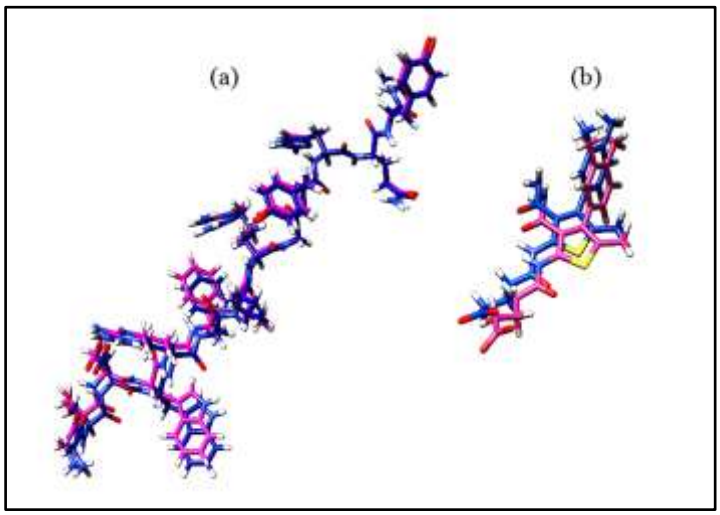

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

The docking study used a 3D structure of 91 epitopes to determine their activity concerning receptors. The rigid body docking method was used, where the conformation of epitope/ligand and receptor were fixed despite the two molecules' altered spatial position and orientation. The rigid body docking is appropriate for complex systems with a high molecular weight, such as peptide-protein complexes. It is a simple technique because it requires a few calculations. The rigid body docking approach produces adequate or even better models for more complexes than flexible docking methods. However, flexible docking methods may achieve higher accuracy for some targets (Chen et al., 2020; Desta et al., 2020; Tao et al., 2020).

Epitope activity for glioma prevention and treatment was determined based on docking results for MHC II (Table 2) and EphA3 receptor (Table 3). Furthermore, the grid score was obtained from the results. The more negative the grid score, the stronger the interaction between epitope and the receptor. The grid score quantifies the intermolecular interactions between a protein and a ligand. The grid score is the total energy in the gas phase, including the van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). E_{vdW} is computed with a protein model that accounts for all its atoms using the Lennard-Jones 9-6 potential (6-9 potential). The calculation of E_{ele} was performed using Coulomb's law, considering a distance-dependent dielectric, $\varepsilon(r) = 4r$ (Abdjan et al., 2023; Balius et al., 2024; Prentis et al., 2022).

Table 2. Results of epitopes docking with MHC II

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
A2	−78.66	19	−52.31	38	−41.24	57	−42.50	76	−41.27
1	−46.39	20	−58.93	39	−34.08	58	−42.66	77	−41.82
2	−50.78	21	−47.42	40	−50.96	59	−45.67	78	−50.89
3	−31.23	22	−46.53	41	−48.02	60	−38.68	79	−44.74
4	−56.99	23	−41.87	42	−62.73	61	−32.67	80	−39.15

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
5	−43.57	24	−40.02	43	−54.16	62	−44.02	81	−42.17
6	−45.07	25	−38.76	44	−49.98	63	−55.83	82	−40.88
7	−48.32	26	−32.41	45	−42.00	64	−52.28	83	−41.64
8	−37.62	27	−39.94	46	−41.66	65	−48.70	84	−42.17
9	−42.46	28	−47.70	47	−34.89	66	−52.29	85	−43.92
10	−48.87	29	−45.17	48	−16.91	67	−42.51	86	−43.43
11	−45.87	30	−42.05	49	−46.91	68	−41.66	87	−37.24
12	−41.79	31	−40.56	50	−42.32	69	−42.91	88	−37.73
13	−42.46	32	−40.64	51	−39.20	70	−41.26	89	−51.31
14	−39.12	33	−49.24	52	−38.96	71	−45.32	90	−38.54
15	−40.34	34	−47.13	53	−44.49	72	−42.15	91	−35.49
16	−50.80	35	−43.49	54	−41.60	73	−48.86		
17	−43.56	36	−44.73	55	−44.52	74	−49.06		
18	−37.73	37	−35.75	56	−40.95	75	−42.32		

Table 3. Results of epitopes docking with EphA3 receptor

Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)	Epitope	Grid Score (kcal/mol)
164	−43.10	19	−46.63	38	−43.85	57	−52.83	76	−34.15
1	−54.11	20	−46.06	39	−31.12	58	−47.88	77	−39.59
2	−50.70	21	−47.97	40	−42.89	59	−33.48	78	−36.57
3	−33.67	22	−48.24	41	−6.95	60	−37.44	79	−48.04
4	−42.39	23	−43.46	42	−42.88	61	−38.44	80	−36.29
5	−43.60	24	−47.67	43	−50.49	62	−43.70	81	−39.78
6	−41.99	25	−42.74	44	−38.64	63	−41.95	82	−45.47
7	−45.66	26	−41.21	45	−31.13	64	−42.06	83	−46.73
8	−28.32	27	−40.52	46	−50.26	65	−50.95	84	−43.64
9	−50.01	28	−47.65	47	−26.08	66	−32.76	85	−43.49
10	−47.53	29	−43.60	48	−4.60	67	−34.22	86	−46.97
11	−49.10	30	−55.52	49	−29.95	68	−30.15	87	−33.65
12	−54.63	31	−47.36	50	−37.56	69	−44.47	88	−44.85
13	−49.05	32	−42.79	51	−36.96	70	−32.71	89	−45.65
14	−54.03	33	−44.77	52	−35.13	71	−39.76	90	−28.76
15	−51.37	34	−48.99	53	−40.50	72	−45.31	91	−36.68
16	−44.43	35	−44.22	54	−55.56	73	−40.34		
17	−45.19	36	−42.68	55	−50.24	74	−42.23		
18	−34.87	37	−40.97	56	−49.05	75	−35.82		

The results of docking with MHC II obtained the most negative grid score of -62.73 kcal/mol with seven hydrogen bonds from epitope 42, as shown in Figure 2a. Although the grid score was less negative than the grid score obtained for redocking the A2 peptide to MHC II, epitope

42 remained likely to be a new candidate prophylactic vaccines for glioma. This was because the A2 peptide was an endogenous sample used in this study only to find the active side of MHC II (Mamedov et al., 2020; Murthy and Stern, 1997; Wang et al., 2022). Meanwhile, the results of docking with the EphA3 receptor showed the most negative grid score of -55.56 kcal/mol with 11 hydrogen bonds on epitope 54, as shown in Figure 2b. This value was more negative compared to the score obtained for redocking compound 164 to the EphA3 receptor.

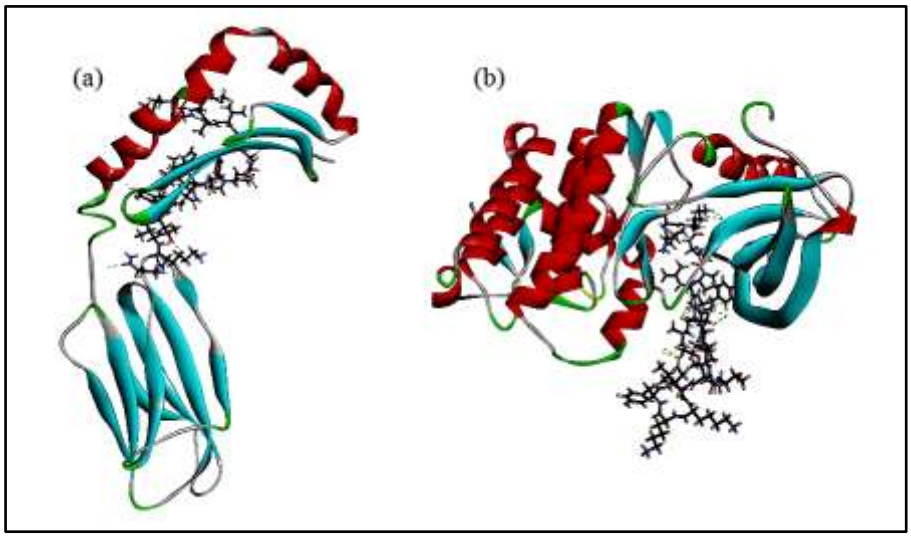

Figure 2. Visualization of docking results: Epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

3.2 Molecular Dynamics Simulations

The molecular dynamics simulations were performed on epitope 42-MHC II and epitope 54-EphA3 receptor complexes. During the process, observation showed that epitope and receptors were flexible (Guterres and Im, 2020; Hashemzadeh et al., 2020; Lazim et al., 2020; Rampogu et al., 2022; Salo-Ahen et al., 2021). Furthermore, the simulations were carried out for 25 ns to determine the stability of docked epitope-receptor complexes.

The stability of epitope-receptor complex could be analyzed from changes in protein structural conformation during the simulation, as indicated by RMSD function and time. Energies and binding interactions between the ligand and protein influence the RMSD value. A protein structure is deemed stable and equilibrated when the RMSD <3 Å (Santha and Vishwanathan, 2022). The simulation of epitope 42-MHC II led to a rapid increase in RMSD at the early stages, namely 17 Å at 1.67 ns. However, after 1.72-12.34 ns, the value decreased to about 4-8 Å. Although there was a major reduction, RMSD> 3 Å showed that the protein was unstable during the process because there are extensive conformational changes (Figure 3). Figure 4 shows the conformation changes of epitope 42-MHC II complex during the simulation.

During the molecular dynamics simulation of epitope 54-EphA3 receptor complex, RMSD fluctuations were stable from the beginning of the process up to 15.29 ns. Subsequently, the value increased drastically to 17 Å and remained stable at 15.48 ns. The results showed that RMSD of epitope 54-EphA3 receptor complex was stable at 1.7–3.4 Å, as shown in Figure 5. At 15 ns in the simulation, the 3D form of epitope 54 changed from a coil to a β-sheet on the last seven amino acids, namely Asn, Thr, Ile, Leu, Lys, Tyr, and Asp, as shown in Figure 6.

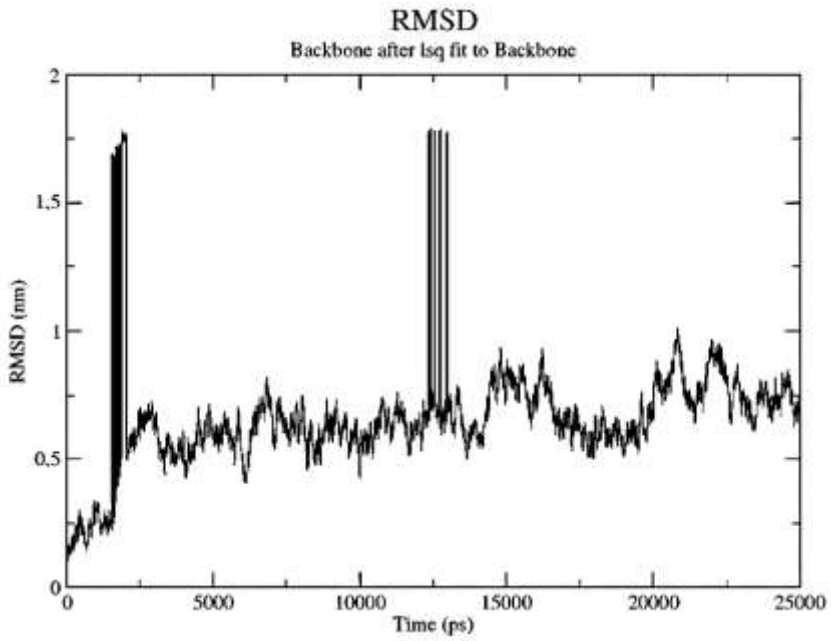

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

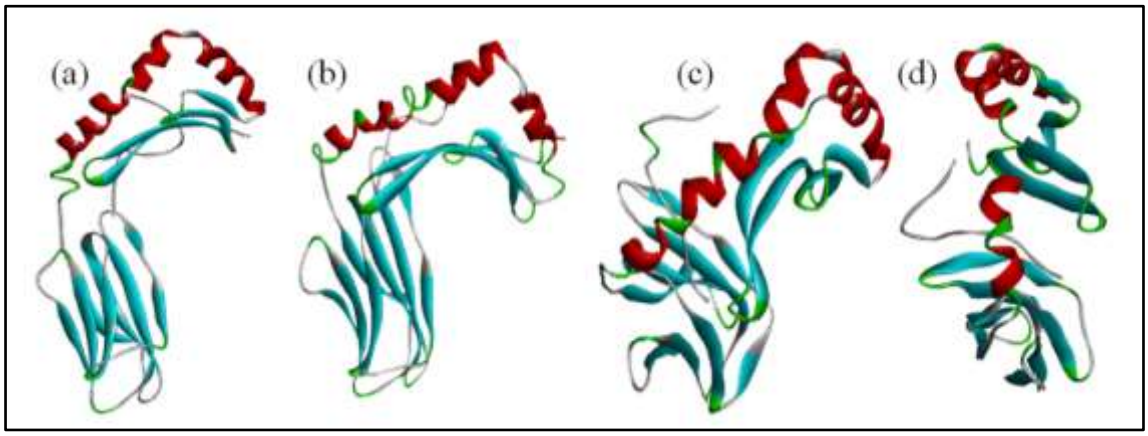

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process, as shown in Figure 7. RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. RMSF quantifies the degree of movement exhibited by each residue throughout a simulation, hence measuring individual residue flexibility. The RMSF of each system member provides information on the movement and stability of each residue in the simulation track. The RMSF graphic illustrates the fluctuation ratio at the residue level, indicating the amino acids in a protein that contribute the most to molecular motion (da Fonseca et al., 2023; Meena et al., 2022; Sargolzaei, 2021). Based on the results, the number of epitope atoms that fluctuated was higher compared to receptors. Atoms of epitope 42 and 54 began from the 3013th and 4450th atomic orders of the complex, respectively.

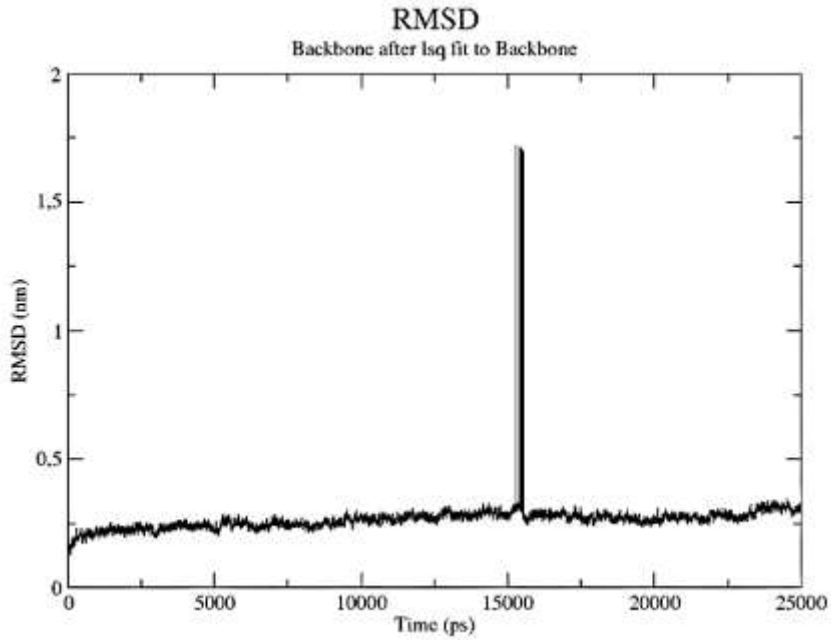

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

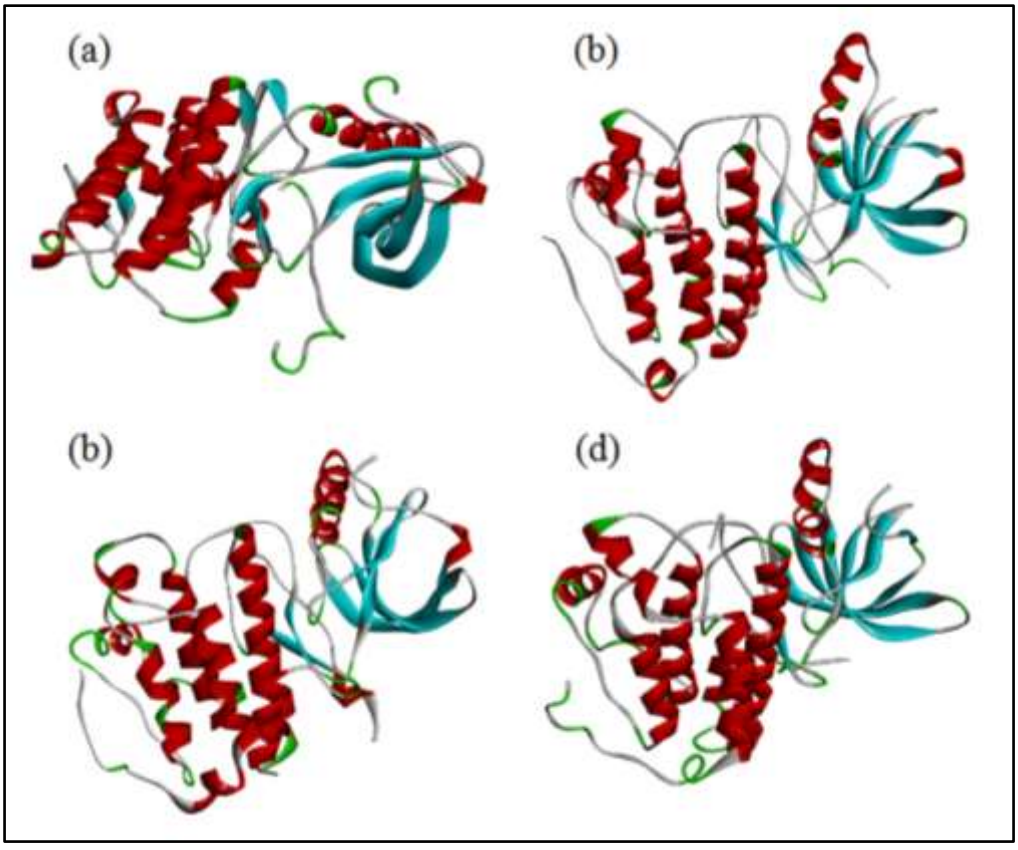

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

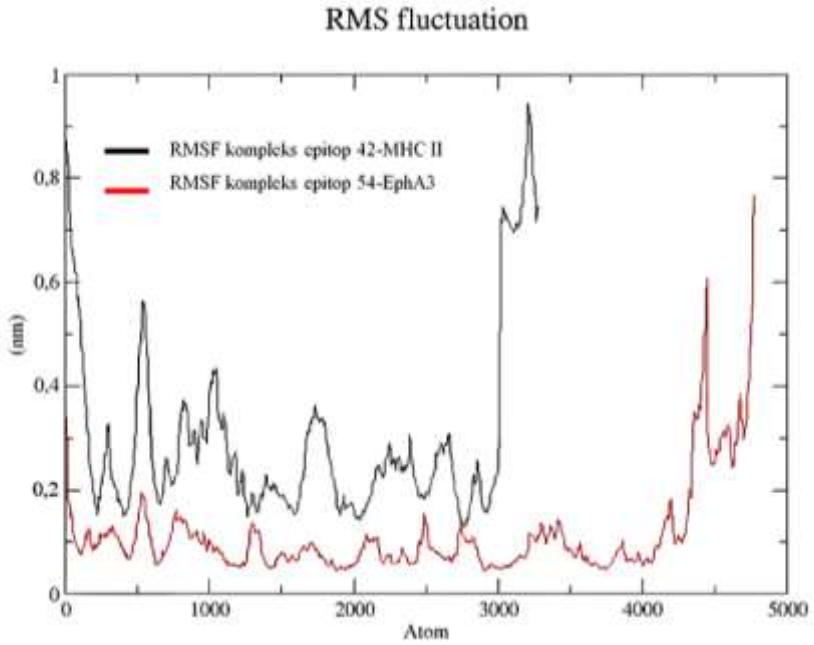

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusions and Recommendation

The best glioma prophylactic and therapeutic vaccines among the 91 epitopes of IDH1 (R132H) were samples 42 and 54, respectively. The grid score of epitope 42 docking into MHC II was -62.73 kcal/mol, while a value of -55.56 kcal/mol was obtained for docking epitope 54 into the EphA3 receptor. During molecular dynamics simulation with a temperature of 300 K, epitope 42-MHC II complex was unstable throughout the process. Meanwhile, the results showed that epitope 54-EphA3 complex was stable from the beginning of the process up to 15.29 ns. Based on these findings, it is important to synthesize epitope 42 and 54 as well as carry out further experimental testing in vitro with the Hs 683 cell line and in vivo to confirm their preventive and curative activity against glioma.

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Re: ECBE1237 - Yeni for Final Layout and Review

USTP MJST <mjst@ustp.edu.ph> Tue, May 28, 2024 at 12:25 PM
To: Yeni Uhamka Klender <yeni@uhamka.ac.id>

Dear Ms. Yeni,

Greetings!

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In Silico Research in Glioma Vaccine Discovery from Isocitrate Dehydrogenase Type 1 (R132H) Epitopes

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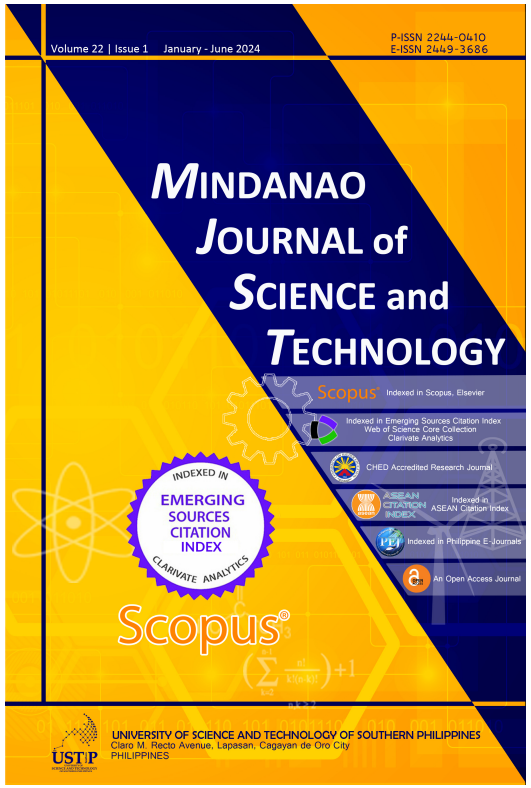
Keywords: *epitope, glioma, IDH1 (R132H), in silico, vaccines*

Abstract

Glioma is a primary malignant brain tumor, which is often detected using the mutation of isocitrate

dehydrogenase type 1 (IDH1) at the R132H position. Several studies have also reported the use of mutated IDH1 (R132H) specific immunogenic epitopes as vaccines against this tumor. Therefore, this study aimed to determine the high-affinity epitopes of IDH1 (R132H) as a plausible candidate of preventive and curative glioma vaccines and to predict the stability of epitope-receptor complex through molecular dynamics simulation. The binding affinity of epitopes for preventing and treating glioma were predicted by docking epitope to major histocompatibility complexes class II (MHC II) and ephrin type-A receptor 3 (EphA3), respectively, using Dock version 6.7. This study used the rigid body docking method, where the samples were treated in their compact state. The highest binding affinity for MHC II was exhibited by epitope 42, as indicated by a grid score of -62.73 kcal/mol. Meanwhile, epitope 54, with a grid score of -55.56 kcal/mol, had the highest binding affinity for the EphA3 receptor. The results showed that the protein conformation in the 42-MHC II epitope complex changed significantly in molecular dynamics simulations using GROMACS version 5.0.6 at 300 K for 25 ns with RMSD > 3 Å, while epitope 54-EphA3 complex was stable from the beginning up to 15.29 ns. Based on these findings, the best candidates for prophylactic and curative



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glioma vaccination were epitope 42 and 54, respectively.

Articles

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