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 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 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 (Ko and Brody, 2021; Choi *et al.*, 2023).

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

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 two 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; 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 (Zheng e*t al*., 2020; Baumgartner *et al*., 2021; Arora *et al*., 2023)

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 (Sunita *et al*., 2020; Rawal *et al*., 2021; Kalita and Tripathi, 2022; Soleymani *et al*., 2022; Guarra and Colombo, 2023).

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; Salo-Ahen *et al*., 2021; Rampogu *et al*., 2022) 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 (Lang *et al*., 2015) based on the method proposed in a previous report (Lang *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 (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; Zhang *et al.*, 2021; Zheng *et al.*, 2022).

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	QYRATDFVV PGPGKV	32	LAFFANALEEVSIE T	63	LVCPDGKTVEAEA AHGTVTR
2	YRATDFVVP GPGKVE	33	DLAACIKGLPNVQ RS	64	VCPDGKTVEAEAA HGTVTRH
3	LVHNFEEGG GVAMGM	34	LAACIKGLPNVQRS D	65	CPDGKTVEAEAAH GTVTRHY
4	HNFEEGGGV AMGMYN	35	AACIKGLPNVQRSD Y	66	PDGKTVEAEAAHG TVTRHYR
5	SIEDFAHSSF QMALS	36	ACIKGLPNVQRSDY L	67	YQKGQETSTNPIASI FAWTR
6	SSFQMALSK GWPLYL	37	TFEFMDKLGENLKI K	68	QKGQETSTNPIASIF AWTRG
7	SFQMALSKG WPLYLS	38	FEFMDKLGENLKIK L	69	KGQETSTNPIASIFA WTRGL
8	MALSKGWPL YLSTKN	39	KLGENLKIKLAQA KL	70	QETSTNPIASIFAWT RGLAH
9	LSKGWPLYL STKNTI	40	SKKISGGSVVEMQ GDEMTRI	71	ETSTNPIASIFAWTR GLAHR
10	KGWPLYLST KNTILK	41	KKISGGSVVEMQG DEMTRII	72	TSTNPIASIFAWTRG LAHRA
11	GWPLYLSTK NTILKK	42	QKVTYLVHNFEEG GGVAMGM	73	STNPIASIFAWTRGL AHRAK
12	WPLYLSTKN TILKKY	43	KVTYLVHNFEEGG GVAMGMY	74	TNPIASIFAWTRGL AHRAKL
13	PLYLSTKNTI LKKYD	44	TYLVHNFEEGGGV AMGMYNQ	75	PIASIFAWTRGLAH RAKLDN

Table 1 continued.

14	LYLSTKNTIL KKYDG	45	VTYLVHNFEEGGG VAMGMYN	76	IFAWTRGLAHRAK LDNNKEL
15	YLSTKNTILK KYDGR	46	SIEDFAHSSFQMAL SKGWPL	77	FAWTRGLAHRAKL DNNKELA
16	HRLIDDMVA QAMKSE	47	FAHSSFQMALSKG WPLYLST	78	AWTRGLAHRAKLD NNKELAF
17	RLIDDMVAQ AMKSEG	48	AHSSFQMALSKGW PLYLSTK	79	WTRGLAHRAKLDN NKELAFF
18	LIDDMVAQA MKSEGG	49	HSSFQMALSKGWP LYLSTKN	80	RAKLDNNKELAFF ANALEEV
19	PDGKTVEAE AAHGTV	50	SSFQMALSKGWPL YLSTKNT	81	AKLDNNKELAFFA NALEEVS
20	DGKTVEAEA AHGTVT	51	SFQMALSKGWPLY LSTKNTI	82	KLDNNKELAFFAN ALEEVSI
21	GKTVEAEAA HGTVTR	52	QMALSKGWPLYLS TKNTILK	83	LDNNKELAFFANA LEEVSIE
22	KTVEAEAAH GTVTRH	53	MALSKGWPLYLST KNTILKK	84	DNNKELAFFANAL EEVSIET
23	ASIFAWTRG LAHRAK	54	LSKGWPLYLSTKN TILKKYD	85	FMTKDLAACIKGLP NVQRSD
24	SIFAWTRGL AHRAKL	55	SKGWPLYLSTKNTI LKKYDG	86	MTKDLAACIKGLP NVQRSDY
25	FAWTRGLAH RAKLDN	56	KGWPLYLSTKNTIL KKYDGR	87	TKDLAACIKGLPNV QRSDYL
26	LDNNKELAF FANALE	57	GWPLYLSTKNTILK KYDGRF	88	SDYLNTFEFMDKL GENLKIK
27	DNNKELAFF ANALEE	58	WPLYLSTKNTILKK YDGRFK	89	DYLNTFEFMDKLG ENLKIKL
28	NNKELAFFA NALEEV	59	PLYLSTKNTILKKY DGRFKD	90	YLNTFEFMDKLGE NLKIKLA
29	NKELAFFAN ALEEVS	60	LYLSTKNTILKKYD GRFKDI	91	FEFMDKLGENLKIK LAQAKL
30	KELAFFANA LEEVSI	61	YLSTKNTILKKYDG RFKDIF		
31	ELAFFANAL EEVSIE	62	VLVCPDGKTVEAE AAHGTVT		

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 and 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 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.

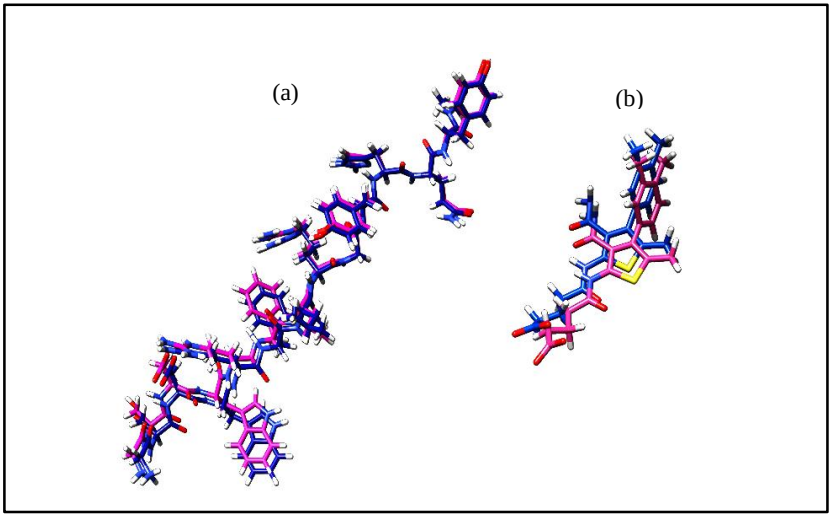

Figure 1. Comparison before (blue) and after (pink) redocking receptors with native ligands: native ligands MHC II, peptide A2 (a), and native ligands EphA3, compound 164 (b)

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 was 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.

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, $\varepsilon (r) = 4r$ (Prentis *et al*., 2022; Abdjan *et al*., 2023; Balius *et al*., 2024).

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 (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 (Murthy and Stern, 1997; Mamedov *et al*., 2020; 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 (Figure 2b). This value was more negative compared with the score obtained for redocking compound 164 to the EphA3 receptor.

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
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		

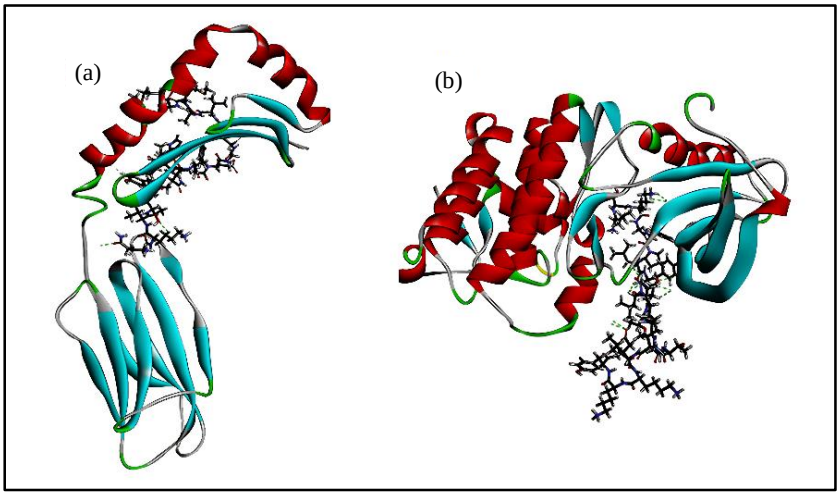

Figure 2. Visualization of docking results: epitope 42 to MHC II (a) and epitope 54 to the EphA3 receptor (b)

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 were 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 (Figure 6).

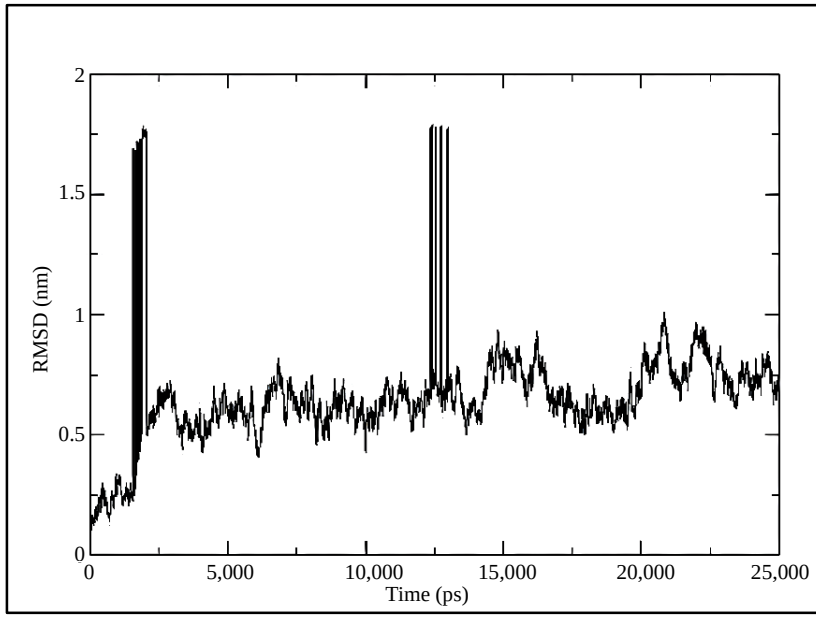

Figure 3. Chart of RMSD changes over time during molecular dynamics simulation of epitope 42-MHC II complex

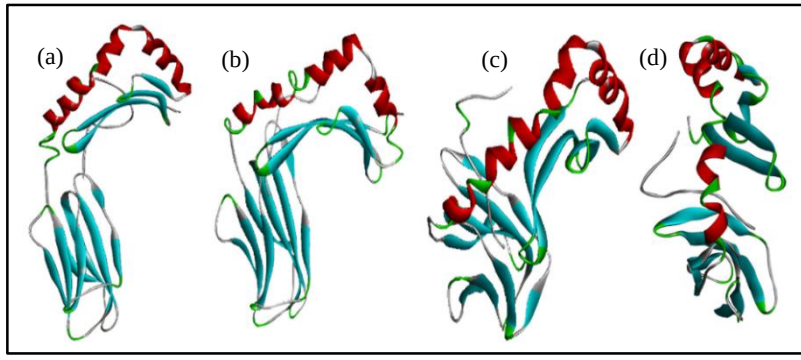

Figure 4. Conformation changes of epitope 42-MHC II complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

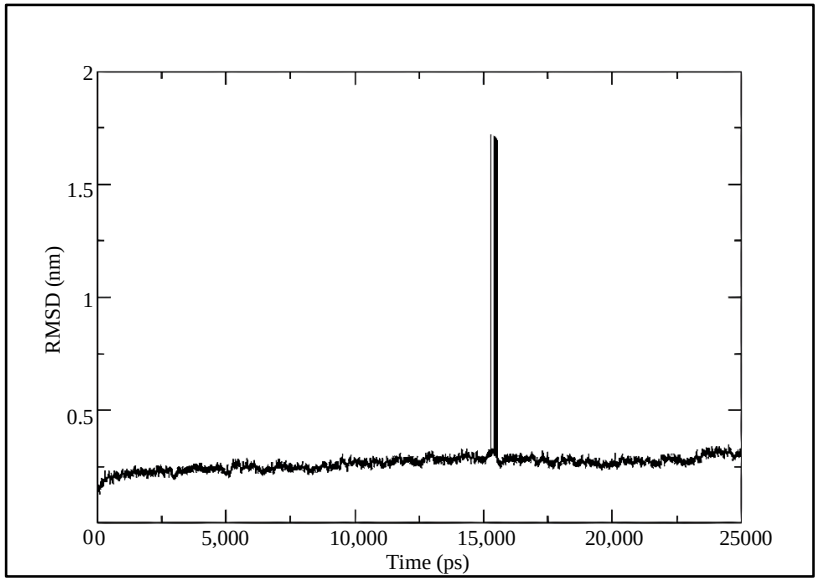

Figure 5. Chart of RMSD changes over time during molecular dynamics simulation of epitope 54-EphA3 receptor complex

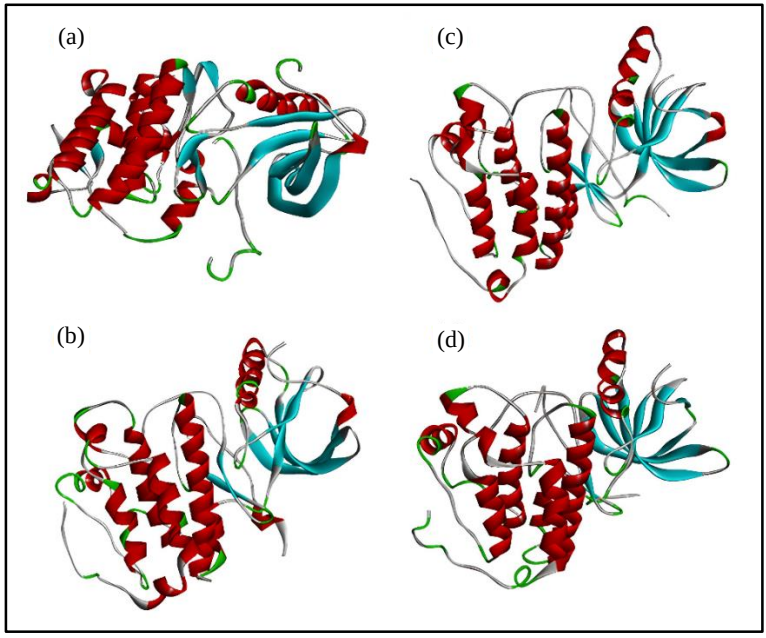

Figure 6. Conformation changes of epitope 54-EphA3 receptor complex during molecular dynamics simulation: time 0 ns (a), time 10 ns (b), time 15 ns (c), and time 25 ns (d)

The movement of atoms in the molecular dynamics simulation of complexes could be analyzed based on the RMSF values obtained during the process (Figure 7). The RMSF value was used to express the average quadratic fluctuation of the minimum distances between proteins and ligands seen in molecular dynamics simulations. The 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 (Sargolzaei, 2021; Meena *et al.*, 2022; da Fonseca *et al.*, 2023). Based on the results, the number of epitope atoms that fluctuated was higher compared with receptors. Atoms of epitope 42 and 54 began from the 3,013th and 4,450th atomic orders of the complex, respectively.

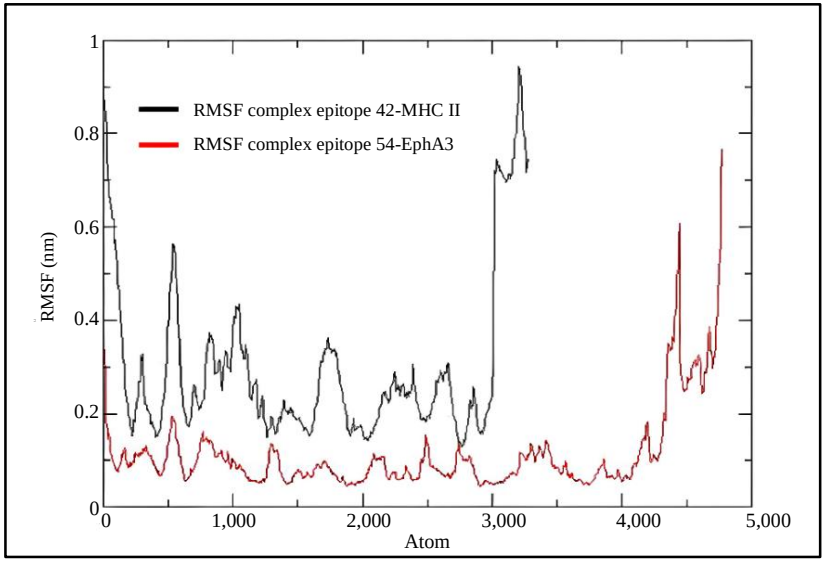

Figure 7. The RMSF chart of molecular dynamics simulations of epitope 42-MHC II complex and epitope 54-EphA3 receptor complex for 25 ns

4. Conclusion 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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