Original Article

Characterization of amino acid residues of T-cell receptors interacting with HLA-A*02-restricted antigen peptides

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Background: The present study aimed to explore residues' properties interacting with HLA-A*02-restricted peptides on T-cell receptors (TCRs) and their effects on bond types of interaction and binding free energy.

Methods: We searched the crystal structures of HLA-A*02-restricted peptide-TCR complexes from the Protein Data Bank (PDB) database and subsequently collected relevant parameters. We then employed Schrodinger to analyze the bond types of interaction and Gromacs 2019 to evaluate the TCR-antigen peptide complex's molecular dynamics simulation. Finally, we compared the changes of bond types of interaction and binding free energy before and after residue substitution to ensure consistency of the conditions before and after residue substitution.

Results: The main sites on the antigen peptides that formed the intermolecular interaction [hydrogen bond (HB) and pi stack] with TCRs were P4, P8, P2, and P6. The hydrophobicity of the amino acids inside or outside the disulfide bond of TCRs may be related to the intermolecular interaction and binding free energy between TCRs and peptides. Residues located outside the disulfide bond of TCR α or β chains and forming pi stack force played favorable roles in the complex intermolecular interaction and binding free energy. The residues of the TCR α or β chains that interacted with peptides were replaced by alanine (Ala) or glycine (Gly), and their intermolecular binding free energy of the complex had been improved. However, it had nothing to do with the formation of HB.

Conclusions: The findings of this study suggest that the hydrophobic nature of the amino acids inside or outside the disulfide bonds on the TCR may be associated with the intermolecular interaction and binding between the TCR and polypeptide. The residues located outside the TCR α or β single-chain disulfide bond and forming the pi-stack force showed a beneficial effect on the intermolecular interaction and binding of the complex. In addition, the part of the residues on the TCR α or β single chain that produced bond types of interaction with the polypeptide after being replaced by Ala or Gly, the intermolecular binding free energy of the complex was increased, regardless of whether HB was formed.

Keywords: Residues of T-cell receptors (residues of TCRs); interaction; HLA-A*02-restricted antigens; residue substitution; TCR-based immunotherapy

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Introduction

T-cell receptors (TCRs) consist of 2 subunits, α, and β, each of which contains a variable region (V region) and a constant region (C region) (1). The V region of the TCR α and β chains contain about 102–109 amino acids, and 3 regions in the V region have highly variable amino acid compositions and sequences known as complementary-determining regions (CDRs). These are as follows: CDR1, CDR2, and CDR3 (2,3). The compositions and sequences of amino acids outside the CDRs are relatively difficult to change and framework regions (FRs), such as FR1, FR2, FR3, and FR4 (4). In the V region, 2 cysteines form disulfide bonds inside the chain to form a cyclic peptide containing about 50–60 amino acid residues, which is similar to the structure and function of the Immunoglobulin variable region (IgV) region and is a domain that specifically recognizes foreign antigens (5,6). TCR chains have chain centrality, that is, the α or β chains of TCRs determine the specificity of major histocompatibility complex (MHC)-restricted antigens, while the corresponding β or α chains could regulate the affinity, activity, or cross-reaction of receptors without affecting antigen specificity (7-9). In the Han population, human leukocyte antigen (HLA)-A02 is the most common HLA-A allele, accounting for half of the population (data based on New allele frequency database, www.allelefrequencies.net) (10). Therefore, cell therapy based on HLA-A02 restricted epitope is more commonly used in the Chinese population (11). The interaction between TCR and potentially immunogenic peptides presented by MHC of antigen-presenting cells (APCs) is one of the most important mechanisms of the adaptive human immune system. A large number of structural simulations of the TCR-peptide-MHC system have been performed, and the large-scale study of the difference in kinetics between free TCR and pMHC-bound TCR is an important research aspect. There are significant differences in the kinetic characteristics of TCR between unbound TCR and TCR-pMHC simulations. Structural biology has demonstrated how T cell receptors bind to the peptide-MHC complex and provides insights into the mechanisms of antigen specificity and cross-reactivity. Related researches have emphasized the influence of structural changes and molecular flexibility. In addition, the analysis of bond types of interactions and binding free energy plays an important role in TCR epitope prediction, antibody-antigen docking and TCR-peptide-MHC modeling (12-15). Docking score, interactions type (bond type and distance) and interaction with amino acids are carried out on a Glide module of Schrodinger Maestro 2019-2 MM Share Version (16-18). Molecular mechanics Poisson Boltzmann surface area (MM-PBSA) is a method of estimating the free energy of interaction, which has been increasingly used in the study of biomolecular interactions (19). GROMACS is used to calculate the relative free energy of protein-ligand binding (20,21). Alanine (Ala) scanning mutagenesis is an experimental technique that sequentially mutates residues in a protein of interest to Ala. The goal is to identify certain residues that contribute the most to the free energy of the interaction. In the study of protein-protein interactions, such residues are usually called “hot spots” (22,23). The function of protein is determined by the interaction between molecules, that is, the interaction between molecules is determined by physical and chemical surface characteristics such as geometry, electrostatic potential, hydrophobicity and concavity (24). Therefore, appropriate amino acid substitutions can produce protein mutants whose activity can be affected by the presence of new ligands through an allosteric mechanism. Glycine (Gly) is the smallest of the 20 natural amino acids. Substituting Gly for amino acids with large side chains seems to be the easiest way to obtain surface cavities. This is confirmed by all available structural analysis protein-small molecule interfaces (25). The protein region partially exposed to the solvent lacks large side chains, which is conducive to close contact with small organic molecules, thereby changing or regulating the activity of the protein (26-28). Given this background, we herein searched the crystal structure of HLA-A*02 restricted antigen peptide-TCR complexes from PDB. We subsequently explored the properties of amino acid residues located inside or outside the disulfide bond of TCR α and β chains interacting with peptides and their effects on bond types of interaction and binding free energy.

Methods

Crystal structure analysis

The PDB was used to search the crystal structures of HLA-A*02-restricted peptide-TCR complexes (http://www.rcsb.org/) (29,30). The following information was collected: PDB ID, HLA allele genotypes, complex names, resolution, and methods. Schrodinger’s theory was applied to obtain the following information: residue closest, distance, specific interactions, hydrogen bond (HB), salt bridges, pi-stacking, and other parameters.

Molecular dynamic simulation optimization

We employed Schrodinger to explore the bond types of interaction of the TCR- antigen peptide complex. In particular, the interaction between TCR and peptides was analyzed. Then, the residues on TCR, which produced intermolecular interactions such as HB and pi-stack, were screened and consequently replaced with Ala and Gly, respectively. After residue substitution, the structures of the proteins were constructed using Schrodinger and preserved as PDB structures. We then obtained protein complexes of residue substitution by pairing the antigen peptide with the protein structure after residue substitution and subsequently analyzing the types of interacting bonds. The resulting docked structures were further refined with molecular dynamics methods. All molecular dynamic analyses were performed with Groningen Machine for Chemical Simulations (GROMACS) 2019 (Development: Herman Berendsen Research Group, Department of Biophysical Chemistry, University of Groningen, Groningen, The Netherlands. Maintenance and update: Science for Life Laboratory, Stockholm University, Stockholm, Sweden). The simulation temperature was set to 300 K, and the all-atomic position of GROMACS and the simple point charge (SPC) water model were selected. The water molecules added around the protein formed a water box simulation system as a periodic boundary for dynamic simulation. In the process of simulation, the Particle-mesh Ewald (PME) algorithm was used. The long-range electrostatic interaction was calculated, and the integration step was set to 2 fs. Under the NVT [canonical ensemble, abbreviated as NVT, means that it has a certain number of particles (N), volume (V), and temperature (T)] ensemble, the system was balanced, and the water was optimized to 500 ps. The NPT [constant-pressure and constant-temperature, abbreviated as NPT, means that it has a certain number of particles (N), pressure (P), and temperature (T)] ensemble was used to balance the system to 500 ps. Finally, molecular dynamics simulations of 20 ns were conducted. Using peptides as ligands and proteins as receptors, the binding force between peptides and proteins was analyzed by Schrodinger. Ensuring that the conditions before and after residue replacement were similar, we compared the changes of bond types of interaction and binding free energy before and after residue replacement (31-34).

Statistical analysis

SPSS 19.0 software is used for statistical analysis, the data of each group is expressed by $\bar{x} \pm s$, and the comparison between multiple groups is analyzed by repeated measures Analysis of variance (ANOVA). P value less than 0.05 is considered to be statistically significant.

Results

Screening of crystal structure of complex

A total of 71 HLA-A*02-restricted peptide-TCR complexes were found from the PDB. Excluding autoimmune antigen peptides and the same crystal structures, 44 different complex crystal structures were screened. The PDB ID, allele genotype, source protein, resolution, and an analytical method for each crystal structure are shown in *Table 1*. The HLA allele genotype of all complexes was HLA-A*02. Most of the complex peptides were derived from viral antigens, such as HTLV-1, influenza M1, HCMV, Epstein-Barr virus, glycoprotein 100, HCV, and HIV p17, as well as tumor-associated antigens (TAAs), such as NY-ESO-1, MART-1, and hTER. The resolution of the crystal structure of all complexes ranged from 1.7 to 3.2 Å. The analytical method of all crystal structures was X-ray diffraction.

*Residue sites on TCRs interacting with HLA-A*02-restricted peptides*

The residue sites of TCRs interacting with HLA-A*02-restricted peptides and the initiation and end sites of corresponding disulfide bonds are shown in *Table 2*. As shown in *Table 2*, sequences referred to as polypeptide sequences, S-start and S-end referred to the beginning and end of disulfide bonds in the α or β chain of TCRs, residue referred to the residues and their positions on the α or β chain of TCRs that interacted with peptides, specific interactions referred to residues and types of interacting bonds (HB and pi stack) of peptides that interacted with TCR, and C referred to the antigen peptides.

The results suggested that the initial and terminal numbers of disulfide bonds in TCR α or β single strands ranged from 21 to 28, and from 87 to 104, respectively. Among the intermolecular interactions formed by TCRs and polypeptides, HBs accounted for 126 of 132, and pi stacks accounted for 6 of 132. Among the residue sites of polypeptides interacting with TCRs; P4 accounted for 45 of 132 (Gly 33/45; Ala 3/45; Asn, Lys, and Met 2/45; Glu, Ile, and Pro 1/45); P8 accounted for 25 of 132 (Tyr 12/25; Thr 6/25; Gln 4/25; Met, Trp, and Val 1/25); P2 accounted

Table 1 Properties of HLA-A*02-restricted peptide-T-cell receptor crystal complexes

No.	PDB ID	HLA allele	Complex description	Resolution (Å)	Method
1	1AO7 (35)	HLA-A2	HTLV-1 TAX	2.6	X-ray diffraction
2	1BD2 (36)	HLA-A2	HTLV-1 TAX	2.5	
3	1QRN (37)	HLA-A2	HTLV-1 Tax (P6A)	2.8	
4	2BNQ (38)	HLA-A2	NY-ESO 157–165-SLLMWITQC	1.7	
5	2GJ6 (39)	HLA-A2	HTLV-1 TAX (Y5K-4-[3-Indolyl]-butyric acid) peptide	2.56	
6	2JCC (40)	HLA-A2	HTLV-1 TAX mutant HLA-A2 W167A	2.5	
7	2PYE (41)	HLA-A2	NY-ESO 157–165 (SLLMWITQC)	2.3	
8	2UWE (40)	HLA-A2	HTLV-1 TAX	2.4	
9	2VLR (42)	HLA-A2	Influenza M1	2.3	
10	3D39 (43)	HLA-A2	HTLV-1 TAX [Y5(4-fluoroPhenylalanine)] peptide	2.81	
11	3D3V (43)	HLA-A2	HTLV-1 TAX [Y5(3,4-difluoroPhenylalanine)] peptide	2.8	
12	3GSN (44)	HLA-A2	HCMV Ag (pp65495–503)	2.8	
13	3HG1 (45)	HLA-A2	MART-1 [1] protein	3	
14	3O4L (46)	HLA-A2	Epstein-Barr virus	2.54	
15	3PWP (47)	HLA-A2	HTLV-1 TAX	2.69	
16	3QDG (48)	HLA-A2	MART-1[26-35](A27L) peptide	2.69	
17	3QDJ (48)	HLA-A2	MART-1[27-35] nonameric peptide	2.3	
18	3QDM (48)	HLA-A2	MART-1[26-35](A27L) decameric peptide	2.8	
19	3QEQ (48)	HLA-A2	MART-1[27-35] nonameric peptide	2.59	
20	4EUP (49)	HLA-A2	MART-1[27-35](A27L) peptide	2.88	
21	4FTV (50)	HLA-A2	HTLV-1 TAX	2.74	
22	4JFE (51)	HLA-A2	MART-1 [1] protein L7A	2.7	
23	4JFF (51)	HLA-A2	MART-1 [1] protein	2.43	
24	4L3E (52)	HLA-A2	MART-1[26-35](A27L) peptide	2.56	
25	4MNQ (53)	HLA-A2	hTERT[540–548] ILAKFLHWL	2.74	
26	4QOK (54)	HLA-A2	MART-1/Melan A26–35 peptide	3	
27	5D2N (55)	HLA-A2	HCMV Ag (pp65495–503)	2.1	
28	5E9D (56)	HLA-A2	MART-1	2.51	
29	5EU6 (57)	HLA-A2	Glycoprotein 100 (gp100)	2.02	
30	5EUO (58)	HLA-A2	Influenza M1	2.1	
31	5HHM (59)	HLA-A2	Influenza M1 influenza A virus (IAV)	2.5	
32	5HHO (59)	HLA-A2	Influenza M1 influenza A virus (IAV)	2.95	
33	5ISZ (60)	HLA-A2	Influenza M1	2.06	
34	5JHD (60)	HLA-A2	Influenza M1	2.46	
35	5JZI (61)	HLA-A2	Hepatitis C virus	2.5	

Table 1 (*continued*)

Table 1 (*continued*)

No.	PDB ID	HLA allele	Complex description	Resolution (Å)	Method
36	5NHT (62)	HLA-A2	MART-1/Melan A26–35 peptide	3.2	
37	5NMG (63)	HLA-A2	HIV p17 Gag-derived	2.75	
38	5TEZ (64)	HLA-A2	Influenza M1 Influenza A virus	1.7	
39	5YXN (65)	HLA-A2	Hepatitis C virus NS3 peptide	2.03	
40	5YXU (66)	HLA-A2	Hepatitis C virus NS3 peptide	2.7	
41	6D78 (67)	HLA-A2	MART-1[27-35](alpha-D26Y,beta-L98W) peptide	2.35	
42	6DKP (67)	HLA-A2	MART-1[26-35](A27L)(alpha-D26Y, alpha-Y50A,beta-L98W) peptide	2.97	
43	6EQB (68)	HLA-A2	MART-1	2.81	
44	6RPB (69)	HLA-A2	NY-ESO-1 157-165	2.5	

for 16 of 132 (Leu 10/16, Ala 4/16, Gly 1/16, Ile 1/16); P6 accounted for 16 of 132 (Val 7/16, Ile 5/16, Gly 3/16, Tyr 1/16); P5 accounted for 11 of 132 (Trp 7/11, Tyr 2/11, Gly and Phe 1/11); P7 accounted for 11 of 132 (Ile 8/11, Thr 2/11, Ala 1/11); P1 accounted for 4 of 132 (Glu 3/4, Lys 1/4); P9 accounted for 3 of 132 (Thr 3/3); P3 was accounted for 1 of 132 (Gly 1/1). Among the amino acid residues on TCR interacting with peptides, Gly accounted for 39 of 132; Ile accounted for 15 of 132; Tyr accounted for 15 for 132; Thr accounted for 11 of 132; Leu accounted for 10 of 132; Ala accounted for 8 of 132; Trp accounted for 8 of 132; Val accounted for 8 of 132; Gln accounted for 4 of 132; Glu accounted for 4 of 132; Lys accounted for 3 of 132; Met accounted for 3 of 132; Asn accounted for 2 of 132; Phe accounted for 1 of 132; and Pro accounted for 1 of 132. TCR and HLA-A*02 had no intermolecular interaction. Even for the same peptides, the residues and sites on the TCRs and the peptides corresponding to the intermolecular interactions were not the same.

Amino acid properties of residues on TCRs interacting with peptides

The amino acid properties of residues on TCRs that formed intermolecular interactions with antigen peptides are shown in *Tables 3* and *4*. *Table 3* shows the residues' amino acid properties located inside the disulfide bond on the single strand of the TCR α chain that formed an HB or pi stack with peptides. The residues were located at positions 29, 30, 31, 37, and 68 of the TCR α chain, and the amino acid properties were mainly polar uncharged (Ser and Gln), which formed HBs with polypeptides. An aromatic amino acid (Tyr) reacted with peptides to form pi stacks, and positively charged amino acid (Arg) reacted with peptides to form HBs. There was a diversity of amino acids at position 31, polar uncharged amino acids (Gln and Ser), and aromatic amino acids (Tyr). *Table 4* shows the residues' amino acid properties located inside the disulfide bond on the single strand of the TCR β chain that formed HBs with peptides. The residues were located at positions 26, 28, 30, 32, 33, 37, 48, and 52 of the TCR β chain. The amino acid properties were mainly polar uncharged (Asn and Gln) and negatively charged (Glu and Asp), which formed HBs with antigen peptides. There were various amino acids at position 28, which could be polar uncharged amino acid (Asn) and negatively charged amino acid (Glu). *Table 5* shows the residues' properties outside the disulfide bond on the TCR α chain that formed HBs or pi stack with peptides. The residues were located at positions 90, 92, 94, 95, 96, 98, 100, 101, 102, and 103 of the TCR α chain. The amino acid properties were mainly polar uncharged (Asn and Ser), non-polar aliphatic (Ala, Pro, Gly), negatively charged (Asp), and aromatic (Tyr). Except for the residues at position 103, all formed HBs with polypeptides. Tyr, at positions 100 and 103, reacted with the antigen peptides to form a pi stack. Moreover, the residues of several binding sites were diverse, and the 94th position could be polar uncharged amino acids (Asn and Ser) and also be non-polar aliphatic amino acids (Ala and Pro). The 96th position was all non-polar aliphatic amino acids (Ala and Gly). The 98th position could be polar uncharged amino acid (Asn), non-polar aliphatic amino acid (Ala), and negatively charged amino acid (Asp). The 100th

Table 2 Residues, sites, and Bond types of intermolecular interaction, between T-cell receptors and peptides

No.	PDB ID	Sequence	S-start	S-end	Residue	Specific interactions
1	3QDJ	AAGIGILTV	22	88	α:30:Gln	1× hb to C:2:Ala 1× hb to C:4:Ile
2	3QEQ		23	91	β:98:Val	1× hb to C:6:Ile
3	6D78		22	88	α:30:Gln	1× hb to C:2:Ala 1× hb to C:4:Gly
4	6EQB		23	89	α:31:Gln	1× hb to C:2:Ala 1× hb to C:4:Gly
			23	91	β:98:Leu	2× hb to C:7:Ile
5	4EUP	ALGIGILTV	23	91	α:30:Gln	1× hb to C:2:Leu
			24	90	β:96:Leu	1× hb to C:8:Thr
			24	90	β:98:Thr	1× hb to C:3:Gly 1× hb to C:5:Gly 2× hb to C:6:Ile
6	2JCC	ALWGFFPVL	22	90	β:97:Ala	1× hb to C:4:Gly
			22	90	β:102:Ser	1× hb to C:4:Gly
7	2UWE		22	90	β:97:Ala	1× hb to C:4:Gly
			22	90	β:102:Ser	1× hb to C:4:Gly
8	4QOK	EAAGIGILTV	23	91	β:98:Leu	2× hb to C:7:Ile
			23	89	α:31:Gln	1× hb to C:1:Glu 1× hb to C:2:Ala 1× hb to C:4:Gly
9	4JFE	ELAGIGALTV	23	89	α:31:Gln	1× hb to C:2:Leu 1× hb to C:4:Gly
			23	91	β:98:Leu	2× hb to C:7:Ala
10	3HG1	ELAGIGILTV	23	89	α:92:Asn	1× hb to C:4:Gly
			23	91	β:98:Leu	1× hb to C:7:Ile
			23	89	α:31:Gln	1× hb to C:4:Gly
11	3QDG		22	88	α:30:Gln	1× hb to C:2:Leu 1× hb to C:4:Gly
12	3QDM		22	88	α:68:Arg	2× hb to C:1:Glu
			23	91	β:96:Val	1× hb to C:9:Thr
			23	91	β:98:Val	1× hb to C:7:Ile
13	4JFF		23	89	α:31:Gln	1× hb to C:1:Glu 1× hb to C:2:Leu 1× hb to C:4:Gly
			23	91	β:98:Leu	2× hb to C:7:Ile
14	4L3E		22	88	α:30:Gln	1× hb to C:4:Gly
			26	94	β:33:Asn	1× hb to C:9:Thr
15	5E9D		23	91	β:98:Met	1× hb to C:9:Thr
			22	88	α:94:Ser	1× hb to C:4:Gly

Table 2 (*continued*)

Table 2 (*continued*)

No.	PDB ID	Sequence	S-start	S-end	Residue	Specific interactions
16	5NHT		23	89	α:31:Gln	1× hb to C:2:Leu 1× hb to C:4:Gly
17	6DKP		22	88	α:30:Gln	1× hb to C:2:Leu 1× hb to C:4:Gly
18	5HHO	GILEFVFTL	25	93	β:52:Gln	1× hb to C:4:Glu 1× hb to C:6:Val
19	2VLR	GILGFVFTL	25	93	β:52:Gln	1× hb to C:4:Gly 1× hb to C:6:Val
20	5EUO		24	90	α:98:Asn	1× hb to C:2:Ile 1× hb to C:4:Gly
			21	89	β:48:Gln	1× hb to C:4:Gly 1× hb to C:6:Val
21	5ISZ		23	90	α:95:Asn	1× hb to C:4:Gly
			25	93	β:32:Asp	1× hb to C:8:Thr
			25	93	β:52:Gln	1× hb to C:4:Gly
22	5JHD		23	92	α:103:Tyr	1× pi stack to C:5:Phe
			25	93	β:32:Asp	1× hb to C:8:Thr
			23	92	α:98:Ala	1× hb to C:4:Gly
23	5TEZ		24	91	α:101:Gln	1× hb to C:6:Val
			23	91	β:99:Trp	1× hb to C:6:Val
24	5HHM	GILGLVFTL	25	93	β:52:Gln	1× hb to C:4:Gly 1× hb to C:6:Val
25	3O4L	GLCTLVAML	24	95	β:101:Thr	1× hb to C:8:Met
26	4MNQ	ILAKFLHWL	23	87	α:94:Ala	1× hb to C:4:Lys
			23	87	α:90:Asp	1× hb to C:4:Lys
			23	91	β:96:Gln	1× hb to C:8:Trp
27	5JZI	KLVALGINAV	28	97	α:102:Asp	1× hb to C:4:Ala 2× hb to C:6:Gly 1× hb to C:7:Ile
28	5YXN		24	93	α:29:Ser	1× hb to C:1:Lys
			24	93	α:98:Asp	1× hb to C:4:Ala 1× hb to C:6:Gly 1× hb to C:7:Ile
29	5YXU		24	93	α:98:Asp	1× hb to C:4:Ala 1× hb to C:6:Gly 1× hb to C:7:Ile
30	3PWP	LGYGFVNYI	23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:30:Gln	1× hb to C:2:Gly 1× hb to C:4:Gly
			22	90	α:100:Ser	1× hb to C:4:Gly

Table 2 (*continued*)

Table 2 (*continued*)

No.	PDB ID	Sequence	S-start	S-end	Residue	Specific interactions
31	3D3V	LLFG [(3,4-difluoro)F] PVYV	23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:100:Ser	1× hb to C:4:Gly
			23	92	β:98:Leu	1× hb to C:8:Tyr
32	3D39	LLFG [(4fluoro)F] PVYV	23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:100:Ser	1× hb to C:4:Gly
			23	92	β:98:Leu	1× hb to C:8:Tyr
33	2GJ6	LLFGKPVYV	23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:30:Gln	1× hb to C:2:Leu 1× hb to C:4:Gly
			22	90	α:100:Ser	1× hb to C:4:Gly
			23	92	β:98:Leu	1× hb to C:8:Tyr
34	1QRN	LLFGYAVYV	23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:30:Gln	1× hb to C:2:Leu
			22	90	β:100:Ser	1× hb to C:4:Gly
			23	92	β:98:Leu	1× hb to C:8:Tyr
			22	90	α:31:Ser	1× hb to C:5:Tyr
35	1AO7	LLFGYPVYV	22	90	α:30:Gln	1× hb to C:2:Leu
			22	90	α:31:Ser	1× hb to C:5:Tyr
			22	90	α:100:Ser	1× hb to C:4:Gly
			23	92	β:98:Leu	1× hb to C:8:Tyr
36	1BD2		23	92	β:104:Tyr	1× pi stack to C:5:Tyr
			22	90	α:96:Ala	1× hb to C:4:Gly
			23	92	β:98:Gly	1× hb to C:8:Tyr
37	4FTV		23	92	β:30:Glu	1× hb to C:8:Tyr
			22	90	α:100:Ser	1× hb to C:4:Gly
38	3GSN	NLVPMVATV	23	91	β:30:Glu	1× hb to C:8:Thr
			23	91	β:97:Thr	1× hb to C:8:Thr
39	5D2N		23	92	β:100:Thr	1× hb to C:6:Val
40	5NMG	SLFNTIAVL	23	89	α:96:Gly	1× hb to C:4:Asn
			23	89	α:94:Asn	1× hb to C:2:Leu 1× hb to C:4:Asn
			23	91	β:96:Thr	1× hb to C:8:Val
41	2PYE	SLLMWITQC	21	89	β:26:Asn	1× hb to C:8:Gln
			21	89	β:96:Asn	1× hb to C:7:Thr
			23	90	α:94:Pro	1× hb to C:5:Trp
			23	90	α:31:Tyr	2× pi stack to C:5:Trp

Table 2 (*continued*)

Table 2 (*continued*)

No.	PDB ID	Sequence	S-start	S-end	Residue	Specific interactions
			21	89	β:94:Leu	1× hb to C:6:Ile
			23	90	α:100:Tyr	1× hb to C:4:Met 1× pi stack to C:5:Trp
			21	89	β:28:Glu	1× hb to C:8:Gln
42	2BNQ	SLLMWITQV	23	90	α:94:Pro	1× hb to C:5:Trp
			22	90	β:97:Asn	1× hb to C:7:Thr
			23	90	α:31:Tyr	2× pi stack to C:5:Trp
			23	90	α:100:Tyr	1× hb to C:4:Met 1× pi stack to C:5:Trp
			22	90	β:95:Val	1× hb to C:6:Ile
43	6RPB		23	104	β:28:Asn	1× hb to C:8:Gln
			23	104	α:37:Gln	1× hb to C:5:Trp
			23	104	β:109:Leu	1× hb to C:6:Ile
			23	104	β:37:Glu	1× hb to C:8:Gln
44	5EU6	YLEPGPVTV	24	91	α:101:Tyr	1× hb to C:4:Pro
			24	93	β:98:Ile	1× hb to C:8:Thr

Table 3 Characteristics of residues inside the disulfide bond of the T-cell receptor (TCR) α chain

Position on the TCR single chain	Amino acid residue	Properties	Bond types
29	Ser	Polar uncharged, non-hydrophobic	HB
30	Gln	Polar uncharged, non-hydrophobic	HB
31	Tyr	Aromatic, non-hydrophobic	Pi stack
	Gln	Polar uncharged, non-hydrophobic	HB
	Ser	Polar uncharged, non-hydrophobic	HB
37	Gln	Polar uncharged, non-hydrophobic	HB
68	Arg	Positively charged, non-hydrophobic	HB

position could be polar uncharged (Ser) or aromatic (Tyr) amino acid. The 101st position could be polar uncharged (Gln) or aromatic (Tyr) amino acids. *Table 6* shows the residues' properties outside the disulfide bond on the TCR β chain that formed HB or pi stack with polypeptides. These residues were located at positions 94–102 and 104–109 of the TCR β chain and were mainly polar uncharged (Asn, Gln, Thr, and Ser), non-polar aliphatic (Leu, Val, Ala, Gly, Ile, and Met), and aromatic (Trp and Tyr) amino acids. Except for the 104th position, they were all formed HBs with the polypeptides. Tyr at the 104th position formed a pi stack with the polypeptide. Moreover, the amino acid residues of several binding sites were diverse. The 96th position could be polar uncharged amino acids (Asn, Gln, and Thr) or non-polar aliphatic amino acids (Leu and Val); the 97th position could be polar uncharged amino acid (Asn and Thr) or non-polar aliphatic amino acid (Ala); the 98th position could be non-polar aliphatic amino acids (Gly, Ile, Leu, Met, and Val) or polar uncharged amino acids (Thr); and the 100th position could be polar uncharged amino acids (Ser and Thr). Also, according to the hydrophobicity of the residues, we found that the

Table 4 Characteristics of residues inside the disulfide bond of the T-cell receptor (TCR) β chain

Position on the TCR single chain	Amino acid residue	Properties	Bond types
26	Asn	Polar uncharged, non-hydrophobic	HB
28	Glu	Negatively charged, non-hydrophobic	HB
	Asn	Polar uncharged, non-hydrophobic	HB
30	Glu	Negatively charged, non-hydrophobic	HB
32	Asp	Negatively charged, non-hydrophobic	HB
33	Asn	Polar uncharged, non-hydrophobic	HB
37	Glu	Negatively charged, non-hydrophobic	HB
48	Gln	Polar uncharged, non-hydrophobic	HB
52	Gln	Polar uncharged, non-hydrophobic	HB

Table 5 Characteristics of residues outside the disulfide bond of the T-cell receptor (TCR) α chain

Position on the TCR single chain	Amino acid residue	Properties	Bond types
90	Asp	Negatively charged, non-hydrophobic	HB
92	Asn	Polar uncharged, non-hydrophobic	HB
94	Ala	Non-polar aliphatic, hydrophobic	HB
	Asn	Polar uncharged, non-hydrophobic	HB
	Pro	Non-polar aliphatic, non-hydrophobic	HB
	Ser	Polar uncharged, non-hydrophobic	HB
95	Asn	Polar uncharged, non-hydrophobic	HB
96	Ala	Non-polar aliphatic, hydrophobic	HB
	Gly	Non-polar aliphatic, non-hydrophobic	HB
98	Ala	Non-polar aliphatic, hydrophobic	HB
	Asn	Polar uncharged, non-hydrophobic	HB
	Asp	Negatively charged, non-hydrophobic	HB
100	Ser	Polar uncharged, non-hydrophobic	HB
	Tyr	Aromatic, non-hydrophobic	HB, pi stack
101	Gln	Polar uncharged, non-hydrophobic	HB
	Tyr	Aromatic, non-hydrophobic	HB
102	Asp	Negatively charged, non-hydrophobic	HB
103	Tyr	Aromatic, non-hydrophobic	Pi stack

Table 6 Characteristics of residues outside the disulfide bond of the T-cell receptor (TCR) β chain

Position on the TCR single chain	Amino acid residue	Properties	Bond types
94	Leu	Non-polar aliphatic, hydrophobic	HB
95	Val	Non-polar aliphatic, hydrophobic	HB
96	Asn	Polar uncharged, non-hydrophobic	HB
	Leu	Non-polar aliphatic, hydrophobic	HB
	Gln	Polar uncharged, non-hydrophobic	HB
	Thr	Polar uncharged, non-hydrophobic	HB
	Val	Non-polar aliphatic, hydrophobic	HB
97	Ala	Non-polar aliphatic, hydrophobic	HB
	Asn	Polar uncharged, non-hydrophobic	HB
	Thr	Polar uncharged, non-hydrophobic	HB
98	Gly	Non-polar aliphatic, non-hydrophobic	HB
	Ile	Non-polar aliphatic, hydrophobic	HB
	Leu	Non-polar aliphatic, hydrophobic	HB
	Met	Non-polar aliphatic, hydrophobic	HB
	Thr	Polar uncharged, non-hydrophobic	HB
	Val	Non-polar aliphatic, hydrophobic	HB
99	Trp	Aromatic, non-hydrophobic	HB
100	Ser	Polar uncharged, non-hydrophobic	HB
	Thr	Polar uncharged, non-hydrophobic	HB
101	Thr	Polar uncharged, non-hydrophobic	HB
102	Ser	Polar uncharged, non-hydrophobic	HB
104	Tyr	Aromatic, non-hydrophobic	Pi stack
109	Leu	Non-polar aliphatic, hydrophobic	HB

residues inside the disulfide bond of the TCR α or β single-chain were all non-hydrophobic amino acids; however, outside the disulfide bond, the residue of the TCR α single-chain located at the 94th, 96th, and 98th positions was Ala, which is a hydrophobic amino acid; the ones of the TCR β single-chain located at the 94th (Leu), 95th (Val), 96th (Leu, Val), 97th (Ala), 98th (Ile, Leu, Met, Val), and 109th (Leu) positions were all hydrophobic amino acids. The above-mentioned hydrophobic amino acids were all non-aliphatic amino acids, which formed HBs with polypeptides.

Molecular dynamic simulation of peptides and TCRs

As shown in *Table* 7, the highest binding free energy of the TCR and 1BD2 interaction was that Tyr at position 104 outside the disulfide bond of TCR β chain formed a pi stack with Tyr at position 5 of the polypeptide, and its binding free energy was –5.62 KJ/mol. However, the lowest binding free energy was that Gly at position 98, located outside the disulfide bond in the β chain, created HB with Tyr at position 8 of the antigen peptides with a binding free energy of 2.43 KJ/mol. The highest binding free energy of the interaction between TCR and 4FTV was Ser at position 100, located outside the disulfide bond of the α chain, which formed HBs with Gly at position 4 of the polypeptide; binding free energy was –2.66 KJ/mol. The lowest binding free energy was Gly at position 30, located inside the disulfide bond of TCR β, which formed HBs with

Table 7 Bond types and binding free energy of antigen peptides and T-cell receptors (TCRs)

PDB ID	TCR single chain	Residues and positions	Intermolecular force with polypeptide	Position on disulfide bond	Binding free energy (KJ/mol)
1BD2	α	ALA-96	1× hb to :4:Gly	Outside	–4.77
	β	TYR-104	1× pi stack to 5:Tyr	Outside	–5.62
	β	GLY-98	1× hb to :8:Tyr	Outside	2.43
4FTV	α	SER-100	1× hb to :4:Gly	Outside	–2.66
	β	GLU-30	1× hb to :8:Tyr	Inside	29.77
5ISZ	α	ASN-95	1× hb to :4:Gly	Outside	8.86
	β	ASP-32	1× hb to :8:Thr	Inside	36.07
	β	GLN-52	1× hb to :4:Gly	Inside	7.17
5JHD	α	TYR-103	1× pi stack to :5:Phe	Outside	–8.70
	α	ALA-98	1× hb to :4:Gly	Outside	–3.25
	β	ASP-32	1× hb to :8:Thr	Inside	31.34

Tyr at position 8 of the polypeptide; its binding free energy was 29.77 KJ/mol. The highest binding free energy of the interaction between TCR and 5ISZ was Gln at position 52, located inside the disulfide bond of the β chain, which formed HBs with Gly at position 4 of the polypeptide; its binding free energy was 7.17 KJ/mol. The lowest binding free energy was Asp at position 32 inside the disulfide bond of the β chain, which formed HBs with Thr at position 8 of the polypeptide; its binding free energy was 36.07 KJ/mol. The highest binding free energy of the interaction between TCR and 5JHD was Tyr at position 103 outside the disulfide bond of the α chain, which formed a pi stack with Phe at position 5 of the polypeptide; its binding free energy was –8.70 KJ/mol. The lowest binding free energy was Asp at position 32 inside the disulfide bond of the β chain, which formed HBs with Thr at position 8 of the polypeptide; its binding free energy was 31.34 KJ/mol. As shown in *Table* 7, the binding sites of polypeptides with the same sequence and their specific TCRs were mostly different. The binding free energy of residues located outside the TCR single chain's disulfide bond to the polypeptide was higher than that located inside the TCR single chain. The binding free energy of antigen peptides and TCR residues to form a pi stack was higher than that of HBs, and the residue of pi stack formation was located at position 5 of the polypeptide, Tyr, and Phe; their corresponding residue (Tyr) of the TCR single chain was at position 104 and 103. Furthermore, the residues were all aromatic amino acids.

Effect of residue substitution on the interaction and binding free energy between polypeptides and TCR molecule

To further understand the residues' influence in *Table* 7 on the polypeptide-HLA-TCR complex's intermolecular interactions, we replaced the 9 non-Ala residues with Ala (except Ala-96 and Ala-98), and 10 non-Gly residues were replaced with Gly (except Gly-98). The bond types of interaction and binding free energy changes after the residue replacement were analyzed (*Tables 8* and *9*). ΔE = EBR – EAR (E was referred to as binding free energy; EBR was referred to as binding free energy before replacing; EAR was referred to as binding free energy after replacing). The higher the negative value, the more important were the residues before a replacement for the complex interaction and binding, while the higher the positive value, it was the opposite. A value of ≤–3 KJ/mol was considered significant. As shown in *Table 8*, after the replacement of Ala, the pi stack force between position 104 outside the disulfide bond of the TCR β chain and Tyr at position 5 of the polypeptide in 1BD2 disappeared, and its binding free energy also decreased, indicating that the original Tyr at position 104 was more favorable for the binding of the complex. Likewise, in 5JHD, the pi-stack force between position 103 outside the disulfide bond of the TCR α chain and Phe at position 5 of the polypeptide also disappeared, and its binding free energy was correspondingly reduced. This

Table 8 Interaction force and binding free energy between polypeptides and T-cell receptor (TCR) molecule after alanine replacement

PDB ID	TCR single chain	Residues and positions	Intermolecular force with polypeptide	Position on disulfide bond	Binding free energy (KJ/mol)	ΔE (KJ/mol)
1BD2	β	ALA-104	Outside	None	–2.5072	–3.1113
		ALA-98	Outside	1× hb to D:8:Tyr	–0.5523	2.9845
4FTV	α	ALA-100	Outside	1× hb to D:4:Gly	–7.3781	4.7205
	β	ALA-30	Inside	None	–0.9011	30.676
5ISZ	α	ALA-95	Outside	None	1.6834	7.1747
	β	ALA-32	Inside	None	–2.6058	38.6748
	β	ALA-52	Inside	None	4.9687	2.2051
5JHD	α	ALA-103	Outside	None	–3.3516	–5.3484
	β	ALA-32	Inside	None	–1.5751	32.9148

Table 9 Interaction and binding free energy between polypeptides and T-cell receptor (TCR) molecule after glycine replacement

PDB ID	TCR single chain	Residues and positions	Intermolecular force with polypeptide	Position on disulfide bond	Binding free energy (KJ/mol)	ΔE (KJ/mol)
1BD2	α	GLY-96	Outside	1× hb to D:4:Gly	–2.9071	–1.8668
	β	GLY-104	Outside	None	–0.8815	–4.737
4FTV	α	GLY-100	Outside	1× hb to D:4:Gly	–2.8989	0.2413
	β	GLY-30	Inside	None	–0.768	30.5429
5ISZ	α	GLY-95	Outside	None	3.6838	5.1743
	β	GLY-32	Inside	None	–1.624	37.693
	β	GLY-52	Inside	None	2.6856	4.4882
5JHD	α	GLY-103	Outside	None	–1.0391	–7.6609
	α	GLY-98	Outside	1× hb to D:4:Gly 1× hb to D:5:Phe	–0.9629	–2.2871
	β	GLY-32	Inside	None	–1.0835	32.4232

suggested that the original position of Tyr at 103 was more beneficial for the combination of complexes. However, the remaining residues on the TCR were replaced by Ala; their binding free energy was increased. We further found that the original residues at these sites might have adversely affected the complex binding. The results after replacing the corresponding non-Gly residues with Gly are given in *Table 9*, while similar findings are also shown in *Table 8*. These findings indicate that the residues (all aromatic non-hydrophobic amino acids) located outside the disulfide bonds of the TCR α or β single-chain formed the pi stack force and had a beneficial effect on the intermolecular interaction of the complex. When Ala or Gly replaced the residues on the single chain of TCR α or β, the complex's intermolecular binding free energy may increase, regardless of whether an HB was formed.

Discussion

T cells used the TCR to search for various polypeptides presented by the MHC molecule with high sensitivity and specificity. Wu *et al.* found that the MHC mainly guided TCR docking in a peptide-independent manner, which determined the initial binding (70). Specifically, peptides dominate the stability and specificity of contacting TCR, affecting T cell activation by adjusting the binding time

of the pMHC-I complex. This functional breakdown of peptide-MHC ligands has been shown that the two-step process of TCR recognition was assisted to effectively scan different peptide-MHC complexes on the cell surface, which made the TCR inherently cross-reacted with different peptides bound to the same MHC. Zhang *et al.* used site-directed mutagenesis to estimate the HLA-A2 side chain's contribution to the binding of the 4 TCRs (71). The findings indicated that these TCRs had a different energy footprint from that of HLA-A2, and no residue was involved in the interaction with all TCRs. MHC side chains' total contribution to the total interaction energy was variable, and the lower limit ranged from 11–50%. The molecular dynamic analysis found that MHC side chains' contribution to transition state complexes was small and variable. Madura *et al.* found that the suboptimal anchor residue at position 2 of the peptide allowed the TCR to pull the antigen peptides from the MHC binding groove, thereby facilitating additional contact with the peptide and the MHC surface (54). Our results suggested that the TCR single-chain had no intermolecular interaction with HLA-A*02. The intermolecular interaction between the TCR and polypeptide was mainly an HB, followed by a pi stack.

Human TCR has a strong ability to combine with polymorphic, positively charged hot spot regions, which are almost exclusively unique to the human HLA-A*02 spiral (72). The TCR bonding process requires hot spots to be embedded, resulting in high energy loss offset by complementary electrostatic interactions. The enrichment of negative charges in the TCR binding loop, especially the germline loop encoded by the TCRVα and Vβ genes, enables this to occur and is related to the restricted localization of TCR on HLA-A2 (73). Yu *et al.* also found that the bicyclic loops of TCR α and β chains contain many charged polar residues (73). The amino acids in the CDR3 loop region located on the TCR α and β chains are significantly different, and each chain has a special role in recognizing the antigen-MHC complex. In the present study, the 98th position inside the disulfide bond of the TCR α chain, which produced the intermolecular interaction force with the HLA-A*02 polypeptide, was a negatively charged amino acid (Asp), and the 28th position inside the disulfide bond of TCR β chain was a negatively charged amino acid (Glu). According to the hydrophobic nature of amino acids, the residues inside the disulfide bond of the TCR α or β single-chain were all non-hydrophobic amino acids, while the residue of TCR outside the disulfide bond at the 94th, 96th, and 98th positions of the α single-chain were Ala (hydrophobic amino acid). The 94th (Leu), 95th (Val), 96th (Leu, Val), 97th (Ala), 98th (Ile, Leu, Met, Val), and 109th (Leu) positions of the β single-chain were all hydrophobic amino acids. Moreover, the above-mentioned hydrophobic amino acids were all non-aliphatic amino acids and formed HBs with polypeptides. It was suggested that the hydrophobic nature of the amino acids inside or outside the disulfide bond on the TCR might be related to the interaction between the TCR and the polypeptide; however, further research is warranted.

HLA class I antigens are combined with non-self or abnormally expressed peptides, and then newly formed HLA-I-peptide complexes are presented to T lymphocytes, playing a key role in immune recognition of transformed and virally infected cells (74). The recognition of TAA by HLA-I restricted CTL cells is the main feature of detecting and destroying malignant cells (75). The discovery and molecular characterization of TAA has changed the field of cancer treatment and introduced a new era of cancer immunotherapy, aiming to improve tumor immunogenicity and T cell-mediated anti-tumor immunity (76). This interaction is usually stabilized by the interaction between the HLA surface and the CDR loops 1 and 2 encoded by the TCR germline, while the selectivity of the antigen peptides is guided by direct interaction with the TCR CDR3 loop. Coles *et al.* elucidated that TCR sensed the first residue of the peptide through the residue Trp-167 on HLA, and this residue acted as an adjustable gateway (77). The substitution of the first amino acid of the polypeptide was estimated to change the side-chain conformation of HLA-Trp-167, thereby canceling the binding to TCR. Tripathi *et al.* found that the β-chain loop of TCR had the smallest change (78), suggesting that the β-domain of TCR could promote the interaction with the antigen. Goyarts *et al.* found that point mutations in certain positions in the CDR3β loop lost the ability to recognize antigen peptides (79), while mutations in other CDR3β positions caused a wide range of antigen recognition patterns on the MHC-peptide surface. This unique recognition ability was generated with minimal changes to the TCR CDR3 loop. These observations indicate that the extensive changes in recognition patterns due to minor perturbations in the structure of CDR3 appeared to be a structural strategy for generating a highly diverse TCR library specific to multiple antigens. Wang *et al.* modified the new TCR by introducing amino acid substitutions in CDR2 and CDR3 regions to improve its activity. As a result, this study showed that the

ability of PBL to recognize tumors after TCR transduction with retroviruses was reduced or unchanged due to the substitution of residues in the CDR2 α region of the TCR β chain. However, some residue substitutions, particularly in the 109th and 112th in α regions of CDR3, can enhance tumor recognition (80). For instance, phenylalanine substitution for tyrosine at residue 109 (109Y-F) and the substitution of Ala or lysine for serine at residue 112 (112S-K or 112S-A) can improve tumor recognition.

Furthermore, it has been reported that the combination of amino acid substitution and retrovirus encoding modified TCR109Y-F/112S-K can give the transduced PBL better tumor recognition ability. Wang *et al.* found that for a given polypeptide, a polypeptide-specific and highly conserved amino acid could always be identified at position 98 of the β loop of TCRCDR3 (81). When the VSV peptide was replaced at position 6, it led to compensatory changes in the 98th amino acid residue of the β loop of TCRCDR3 and the CDR3 β chain's length. It was suggested that the 98th amino acid residue of CDR3VSV/H-2kb was the key residue that determines the specificity of the TCR-VSV/H-2kb interaction, and a CDR3β loop of a specific length was required to promote this interaction (81). Zhang *et al.* observed that, in VSV8-specific CTL, position 93 of the α chain of CDR3 was a highly conserved residue (82), and certain substitutions of residues at position 4 of the polypeptide caused a change in position 93. It was indicated that there might be an interaction between the CDR3 α chain and the 4th position of the polypeptide. The replacement of position 6 of VSV8 with a negatively charged residue also resulted in the change of position 93 of the CDR3α chain to a positively charged residue, which indicated that the CDR3α chain might interact with position 6 in some cases. In this study, we found that the positions and residues of HLA-A*02 polypeptides that formed intermolecular interactions with TCR α or β chains were mainly P4 (Gly, Ala), P8 (Tyr, Thr, Gln), P2 (Leu, Ala), and P6 (Val, Ile, Gly). Subsequently, changing the amino acids in the above positions may affect the binding of HLA-A*02 polypeptides to TCR. To confirm this speculation, further experiments were needed. In this regard, we used Ala and Gly to replace the residues on the TCR that interacted with the polypeptide. Our result revealed that the residues (both aromatic and non-hydrophobic) located outside the TCR α or β single-chain disulfide bond and forming the pi-stack force exhibited a beneficial effect on the interaction and binding of complex molecules. Besides, after the residues on the TCR α or β single chain that produced types of interacting bondss with the polypeptide were replaced by Ala or Gly, the intermolecular binding free energy of the complex was increased, suggesting that it was more favorable for the combination of the two. We also observed that this combination had nothing to do with the formation of HB. In summary, this study provides a certain preliminary basis for the difficulty of how to modify TCR through residue substitution to increase the binding of TCR to HLA*A02 restricted antigen. However, its underlying mechanism is still unclear and thus requires further exploration.

Conclusions

In summary, the findings of this study suggest that the residue sites of HLA-A*02-polypeptides that formed HB and pi-stack with TCR, were primarily P4, P8, P2, and P6. The hydrophobic nature of the amino acids inside or outside the disulfide bonds on the TCR may be associated with the intermolecular interaction and binding between the TCR and polypeptide. The residues located outside the TCR α or β single-chain disulfide bond and forming the pi-stack showed a beneficial effect on the intermolecular interaction and binding of the complex. We also noted that part of the residues on the TCR α or β single chain that produced bond types of interaction with the polypeptide after being replaced by Ala or Gly, the intermolecular binding free energy of the complex was increased, regardless of whether HB was formed. Finally, this study provides a valuable theoretical basis for how to modify the TCR, that is, to improve the affinity and stability between the TCR and antigen peptides, and further to promote the binding between the two under the premise of ensuring the specificity of the antigens.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-21-835). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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