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Immunoglobulin interface redesigning to enhance lebrikizumab mediated immunomodulation of IL-13 hyper-response

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ABSTRACT

The overexpression of interleukin-13 (IL-13) leads to autoimmune and inflammatory diseases. These adverse responses can be neutralized by using lebrikizumab as a therapeutic monoclonal antibody (mAb). Herein, we have attempted to modulate the lebrikizumab mAb to enhance its binding affinity towards IL-13. The interface residues of the lebrikizumab-IL-13 complex were determined by the PyMOL and verified by the artificial neural network-based B-cell epitope prediction server (ABCpred server) and the Paratome web server. The Cologne University Protein Stability Analysis Tool (CUPSAT) web server based mutational approach was used to identify the stable and favorable interface mutations in the lebrikizumab. Only 40 mutations were selected to generate a single mutant library, and their binding affinity for IL-13 was analyzed by using the Z-Dock server. Based on high Z-score, mutants having a better affinity with IL-13 were selected to create a multi-mutant library. The multi-mutant library was again subjected to the Z-Dock server, and their binding affinity was determined. The highest-scoring ten mAb mutants were validated by using PatchDock and ClusPro servers. The best two potential mAb mutants were identified and subjected to molecular dynamics (MD) simulations to ensure its structural stability at the microscopic level. The changes in the different bonds as the effect of mutation were assessed by LigPlot + v2.1. The AllerTOP and ToxinPred web servers were used to analyze the non-allergic and nontoxic nature of the selected mutants. Therefore, these redesigned mAb could be used for potential treatment against IL-13 associated diseased conditions.

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Introduction

Cytokines play an important role in developing an immune response against the foreign pathogen, but their dysregulation leads to various inflammatory diseases like rheumatoid arthritis, asthma, multiple sclerosis, etc. (Rossi et al., 2011; Tokayer et al., 2002; Tsai & Tsai, 2017; Wills-Karp & Chiaramonte, 2003). Interleukin-13 is a versatile cytokine mainly secreted by the type-2 helper T-cells, and also by Natural Killer T cells, mast cells, and eosinophil. It plays a central role in IgE synthesis, fibrosis, airway-hypo responsiveness, and Goblet-Cell-hyperplasia (Rogers, 2002), and its dysregulation plays a pivotal role in allergy and asthma (Rael & Lockey, 2011). IL-13 promotes goblet cell differentiation, which further increases the secretion of mucin, which blocks the tracheal airway (Fahy, 2002). It is also involved in the development of insulin resistance pathogenesis (Martínez-Reyes et al., 2018). It acts as a potent inducer of fibrogenesis in chronic Schistosomiasis and atopic dermatitis (Gieseck III et al., 2016). There are two receptors which counter the action of IL-13 named as IL-13R α 1 and IL-13R α 2 (Figure 1).

The later has a higher binding affinity for IL-13 than the former; still, it is not involved in the signaling (decoy) and considered as a neutralizer due to its efficient internalization from extracellular spaces (Luzina et al., 2012).

To manage the overexpression of IL-13 and to minimize its immune-pathogenic roles, monoclonal antibodies play a crucial role via neutralization (Kang et al., 2015; Rider et al., 2016). A literature survey revealed that five monoclonal antibodies are under clinical trials to neutralize the effect of IL-13. These mAbs are CNTO5825; IMA-026; IMA-638; Tralokinumab and Lebrikizumab (Gauvreau et al. 2011; Piper et al., 2013; van Hartingsveldt et al., 2013). Among them, IMA-026, Tralokinumab, and CNTO5825 target the binding of IL-13 to IL-13R α 1/IL-13R α 2, whereas IMA-638 binds to those IL-13 epitope region having an affinity for IL-4R α receptor blocking the downstream STAT6 signaling (Nakamura et al., 2017; Ultsch et al., 2013). Lebrikizumab is an IgG4 humanized mAb which shares the same binding site on IL-13 that shares with the IL-4R α . As the former has a higher affinity for IL-13, it results in inhibition of IL-4R α binding to the IL-13, inactivating IL-13 signaling (Corren et al., 2011). Asthmatic patients

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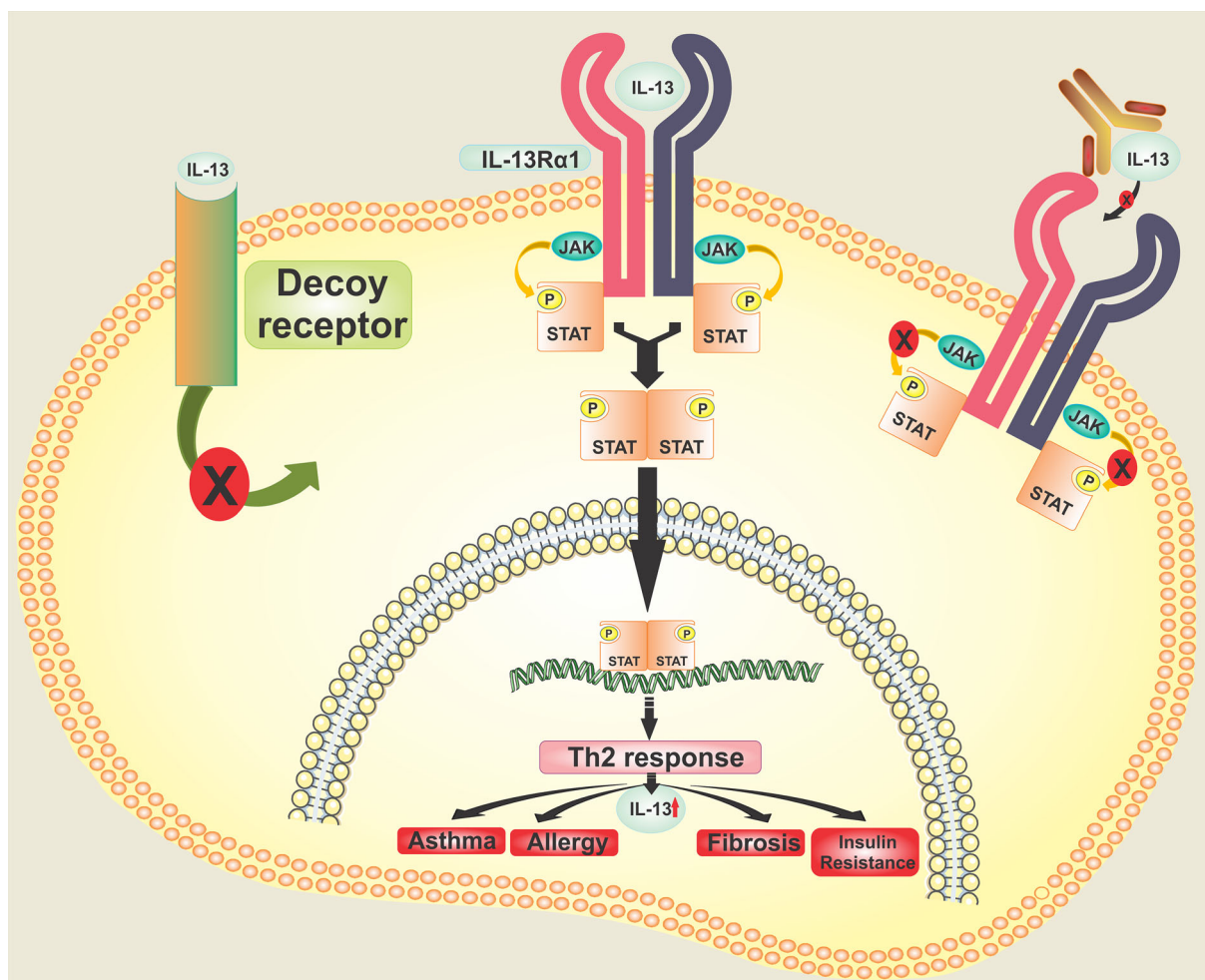


Figure 1. Mechanism action of IL-13 signaling. When IL-13 binds to the IL-13R α 1 receptor and activates JAK-STAT signaling, which mediates the Th2 response leading to Th2 response resulting in inflammatory diseases. Blocking this interaction by mAb inhibits the downstream signaling.

administered with lebrikizumab along with corticosteroid treatment have indicated improved lung functioning and reduced concentrations of Th2 chemokines (CCL13 and CCL17) and IgE. In LAVOLTA1 trials of lebrikizumab for asthma, reduced periostin concentration was observed, but in LAVOLTA 2 trials, there was no significant reduction of periostin exacerbation rates (Nakamura et al., 2017). Although it also indicates some musculoskeletal side effects.

To improve the therapeutic response of lebrikizumab towards IL-13, here, we have utilized a computational mutagenesis approach to increase the affinity and specificity of lebrikizumab for IL-13. The redesigned antibody will require a low dose and will provide faster relief against allergic responses and inflammatory diseases. This will not only overcome the adverse effects of IL-13 but will also inhibit IL-4 receptor signaling via IL-13 blockade at the binding site of this receptor. Here we have used three different docking servers to validate the post mutation stability of the lebrikizumab-IL-13. Based on the docking score, the top two antibodies were sorted as the potential antibodies against IL-13 and subjected to MD simulation (Figure 2). The antibodies with a higher potency can be further used for *in vitro* and *in vivo* experimental validation for their efficacy. This approach opens new gates for finding better cures for inflammatory and autoimmune diseases.

Methodology

Retrieval of lebrikizumab-IL-13 complex and its characterization

The crystal structure of lebrikizumab Fab bound to IL-13 in a complex (Ultsch et al., 2013) was retrieved from the protein data bank (PDB ID:4I77) (<https://www.rcsb.org/structure/4i77>). The primary purpose of this study was to identify the amino acid residues that can be utilized to perform the mutational study to increase the affinity of mutant lebrikizumab for IL-13. For this purpose, we need to know the actual interacting amino acid residues present at the interface of the lebrikizumab-IL-13 complex and, therefore, the tertiary structure of the lebrikizumab-IL-13 complex was separated by using PyMOL. Post-separation lebrikizumab and IL-13 were individually used to determine the paratope and epitopes, respectively. The binding of the antibody with antigen is highly specific, and the residues participate in this binding are basically complementarity-determining regions (CDRs) as predicted by various antigen-binding region prediction tools. However, as per the literature, approximately 22% of residues that actually participate in antigen binding as paratope residues fall outside the traditionally defined CDRs.

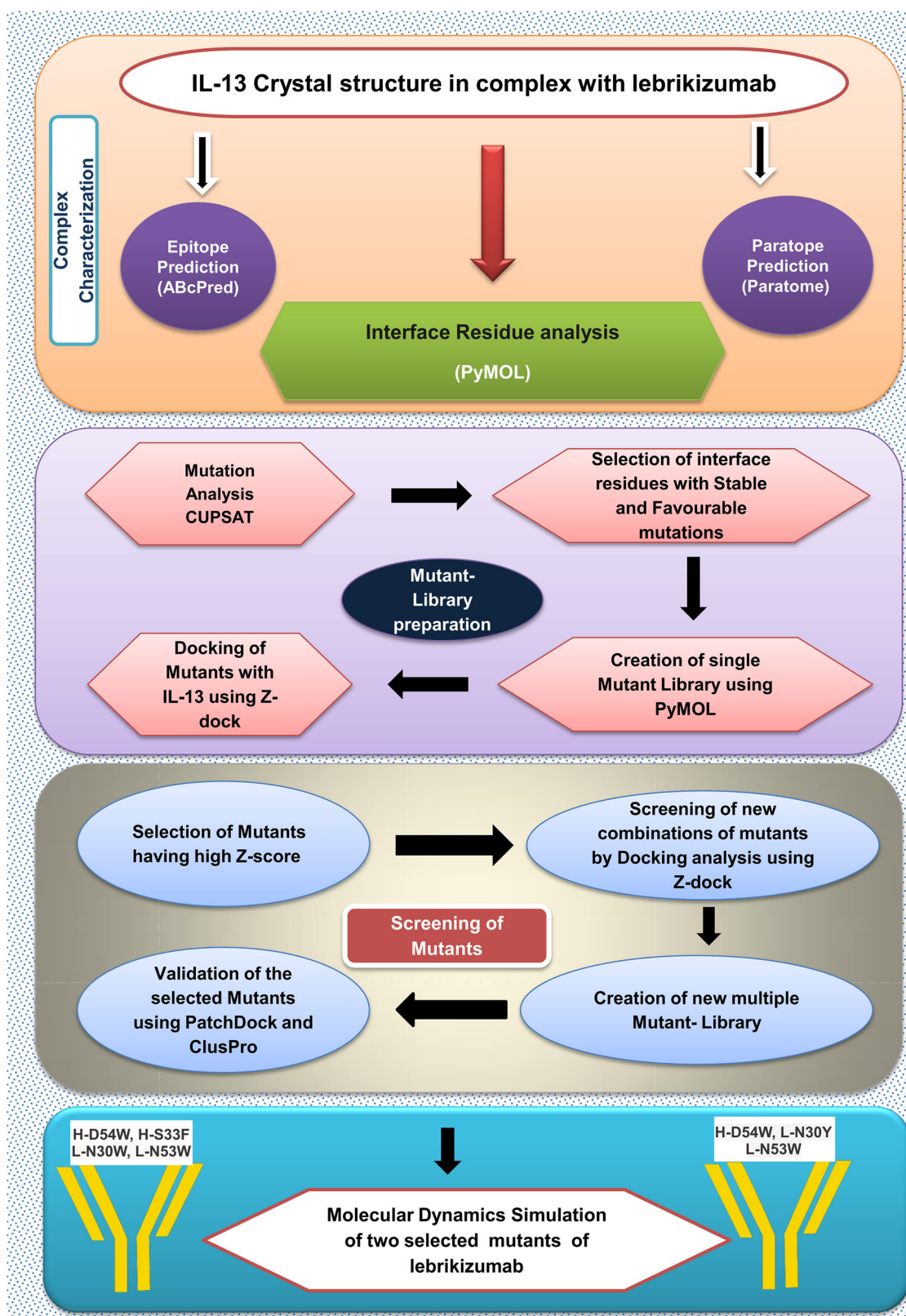


Figure 2. Flow chart for the redesigning of lebrikizumab monoclonal antibody.

The actual interacting interface residues of the lebrikizumab-IL-13 complex were identified and characterized by using the PyMOL molecular graphics tool (DeLano, 2002). The interface residues of lebrikizumab were categorized as CDRs, while the same identified for IL-13 was categorized as epitope residues. However, to identify the actual antigen-

binding residues, i.e. paratope, Paratome web servers (<http://www.ofranlab.org/paratome/>) was utilized, and the identified key residues were used for the mutational study. This server is based on an algorithm that identifies the antigen-binding region from the sequence of amino acids or 3D crystal structure of an antibody. It utilizes the structural consensus

regions within a multiple structure alignment of a non-redundant set of available Ag-Ab complex. This server has the ability to identify 94% of the antigen-binding residues as paratope correctly (Chen & Weng, 2003; Kunik et al., 2012). Later, the ABCpred (Artificial neural network-based B-cell epitope prediction server) web server (<https://bio.tools/abcpred>) was utilized to validate the interface residues of IL-13 as antigenic epitope residues participating in complex formation with lebrikizumab. The FASTA sequence of the IL-13 was used as an input. This server utilizes the artificial neural network and calculates the sensitivity and specificity of amino acids having the correct probability of being in epitope regions. This server assigns a score of 0–1, where 1 represents the higher authenticity of the predicted epitope region and vice-versa (Saha & Raghava, 2006).

Creation of a single mutant library of lebrikizumab

The stability and free energy of all possible mutations in the whole antibody sequence were predicted by using the CUPSAT web server (cupsat.uni-koeln.de) (Parthiban et al., 2006). The segregated lebrikizumab 3D model was subjected to the aforementioned server to evaluate the stability and free energy of all mutations in the whole antibody sequence by the replacement with the other 19 natural amino acids. This server utilizes rigorous algorithms that use amino-acid atom mean force potential and torsion angle distribution to assess the environment of the mutation site and its solvent accessibility (Parthiban et al., 2007). It predicts $\Delta\Delta G$, the difference in free energy of unfolding between wild-type and mutant proteins. The predicted $\Delta\Delta G$ values may be positive or negative, indicating the stabilizing or destabilizing effects of different mutants, respectively (Parthiban et al., 2006). The resulting mutants were sorted based on their presence among the interface residues having stabilized mutation and favorable nature. PyMOL mutagenesis tool was used to execute the CUPSAT resulting mutation in the lebrikizumab, and a single mutant library was created. Each mutant of the library was generated with an individual PDB structure.

Assessment of binding affinity of the single and multi-mutant library of lebrikizumab with IL-13

As we already had a single mutant library, it was necessary to screen those mutants having a better affinity for the IL-13 as compared to the wild type (lebrikizumab without any mutation). Z-dock web server (<http://zdock.umassmed.edu/>) was utilized for this screening. Previously extracted PDB structures of IL-13 and single mutant antibody library was being used as inputs to the Z-Dock web server, and their individual binding affinity score was predicted. This server uses a fast Fourier transform algorithm to find a spatial degree of freedom between receptor and ligand and analyze their binding mode with an energy scoring function called Z-score. Higher the Z-score value more excellent will be the binding affinity for protein-protein complex (Chen & Weng, 2003; Mintseris et al., 2007). The mutants which have higher Z-score as compared to wild type were selected as stable

mutation at the particular amino acid position. All these single mutants with higher Z-score were differently combined to make doublet, triplet, and quadrat mutant library of the lebrikizumab. This multi-mutant library was again subjected to the Z-dock server to determine their binding affinity for IL-13, and the top-scoring mutants were sorted from the whole library based on their Z-dock score.

Validation of binding affinity of top-scoring multi-mutants

The top-scoring multi-mutant, i.e. doublet, triplet, and quadrat, were further screened for the best possible binders using two docking servers, namely PatchDock and ClusPro. The former is a geometry-based molecular docking server that aims to find different transformations that yield good molecular shape complementarity. This server provides dot surface representation of the molecule and also interfaces areas (Connolly, 1983; Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). It gives the geometric score, desolvation energy, the interface area size, and actual rigid transformation (Zhang et al., 1997). The PatchDock server output was further validated by using ClusPro v2.0 (<https://cluspro.bu.edu/login.php>). This server works on the PIPER algorithm and predicts the rank on the stability level, interaction potential, semi-definite programming-based underestimation (SDU) energy, and cluster size (Comeau et al., 2004; Kozakov et al., 2010; Verma et al., 2016). The both PatchDock and ClusPro v2.0 web servers used the PDB structure of the antigen (IL-13) and antibody (mutants) as their input. The top-scoring multi-mutants were sorted based on their scoring on PatchDock and ClusPro servers and subjected to the next step of the study mentioned below.

MD Simulation to verify stable interaction

MD simulation is a unique approach to validate the microscopic interactions present between two biomolecules. Here, we attempted the same to validate the binding affinity between the mutant-antibody and IL-13 molecule. Wild type lebrikizumab was also taken into consideration as a positive control for the comparison purpose. The MD simulation was attempted by using the Gromacs v5.0 standalone tool. GROMOS96 43A1 force field and the particle mesh Ewald summation method were used to run full system MD simulation (Ali et al., 2017; Karplus, 2002). Initially, protein topology was constructed using the Pdb2gmx command. It gives an idea about the characteristics of the bonded and non-bonded regions of the complex. Chloride ions were utilized for the system neutralization. In prior to simulation, the steepest descent algorithm was used to perform the energy minimization to confirm that the geometry of the system is appropriate, and there are no steric clashes in the complex (Jaidhan et al., 2014). The leapfrog algorithm was used to perform the system equilibration. During this step, the temperature of the system was increased up to 300 K and pressure up to 1 bar. Trajectory analysis of the MD simulation was attained for 20 ns. Respective root-mean-square

Table 1. List of interacting residues of heavy and light chain with IL-13.

Heavy chain	IL-13	Light chain	IL-13
F27	C29		
S30	N30	Y28	E12
A31	S32	N30	E61
Y32	I52	F32	K62
S33	N53	Y49	R65
W52	V54	S52	M66
G53	S55	N53	S68
D54	G56	N91	G69
K56	C57	N92	P97
D95	S58	R96	K74
G96	A59		V75
V97	I60		S76
Y98	E61		
P99	Q64		
Y100	R65		
	S68		
	V75		
	A77		

deviation (RMSD) and root-mean-square fluctuation (RMSF) plot were obtained for the comparative stability assessment.

Assessment of bonding pattern change post mutation

To determine the changes in the bonding interactions pattern leading to an increase in binding affinity of mutant-mAb with IL-13 as compared to the wild type lebrikizumab, LigPlot + v2.1 software was used. Initially, the complex file of mAbs (WT & mutants) and IL-13 was exported in .pdb format to be used as an input for the LigPlot+ 2D visualization tool to determine the change in hydrophobic interactions and H-bonding. To know the difference in bonding as the effect of mutation for the enhancement of binding affinity, only those residues were visualized that were mutated. From this, the changes in the number of different bonds on the mutations have been analyzed.

Allergenicity and toxicity prediction of the mutated lebrikizumab

To check the allergic or non-allergic nature of the mutated antibody, it was analyzed by using a freely accessible online tool named as AllerTOPv 2.0 (<https://www.ddg-pharmfac.net/AllerTOP/>). This server is based on the amino acid descriptors, residue hydrophobicity, helix, and β -strand forming propensities, size, and abundance. The protein sequences were transformed into uniform vectors by auto- and cross-covariance, and a machine learning method using k-nearest neighbors was used to classify allergens and non-allergens (Dimitrov et al., 2014).

The designed mutated antibody must be nontoxic in nature, and we assured the same by using the ToxinPred server (<http://crdd.osdd.net/raghava/toxinpred/>). It allows us to generate all possible overlapping peptides and their single mutant analogs of the submitted protein and predict their toxicity. This server has a massive data of peptides from Swissprot and TrEMBL and uses model-based learning technique to overlap the toxicity of different peptides and finally predict the toxic or nontoxic nature of the submitted protein (Gupta et al., 2013).

Physicochemical characterization of the mutated lebrikizumab

We further studied the different physicochemical properties of the mutated antibody using the ProtParam tool (<https://web.expasy.org/protparam/>) (Bjellqvist et al., 1993) and compared with the wild type lebrikizumab. The predicted parameters involve molecular weight, aliphatic index, half-life, theoretical pI, and instability index. It predicts *in-vivo* half-life by using "N-end rule," which describes that the degradation of protein is determined by N-terminal amino acids (Varshavsky, 1997). The instability index of protein was also predicted, and if its value is less than 40, then the protein will be stable, and vice-versa (Gasteiger et al., 2005).

Results

Interface analysis of Lebrikizumab-IL-13 complex

The tertiary structure of lebrikizumab Fab bound to IL-13 in a complex was retrieved from the Protein Data Bank (PDB ID: 4I77). It is a trimer consisting of IL-13 bound to FAb arms of lebrikizumab. The IL-13 composed of 112 amino acid residues and lebrikizumab heavy chain, and the light chain comprises 219 and 218 amino acid residues, respectively. The interface residues of the lebrikizumab and IL-13 complex was determined by the PyMol interface script, revealed that fifteen amino acid residues of the heavy chain (Table 1) interacting with eighteen amino acid residues of IL-13, and nine amino acid residues of light chain interact with eleven IL-13 amino acid residues (Figure 3).

The individual validation of the paratopes of the lebrikizumab (mAb) was performed by the Paratome web server. We found that a total of six paratope regions were identified (Figure 4) in the whole antibody, specifically three paratopes in heavy chain and three in the light chain. Heavy chain paratope spans from amino acid residues 27–35 (ABR1); 47–59 (ABR2); and 97–107 (ABR3), while light chain paratope spans from the amino acid residues 27–107 (ABR1); 50–60 (ABR2) and 93–100 (ABR3). From this validation, we have found all possible amino-acid residues which can be a part of the paratope region and may participate in the interaction with antigen (IL-13).

Similarly, the individual interface analysis of IL-13 was validated by the ABCpred web server and found that nine peptide sequences with their propensity score, indicating the high probability of being a humoral epitope (Table 2). Here we have found that apart from the interface residues which were predicted earlier by PyMOL, there are also some other amino-acid residues that have a probability of participating as the possible epitope region of the IL-13.

Mutation and stable mutant analysis

The mutational analysis of the lebrikizumab, to identify the advantageous mutations has been performed by using CUPSAT web server. This server has substituted every interface amino acid residue with the other 19 residues and generated a large number of combinations (8133) with predicted

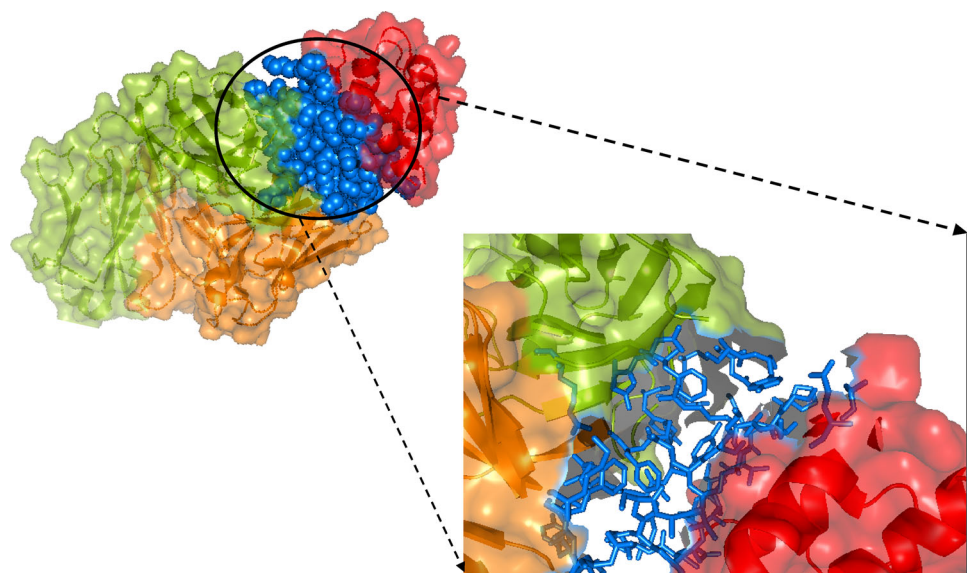


Figure 3. The complex structure of IL-13(red) and lebrikizumab (green and yellow) indicating the interacting residues (blue).

Paratope of heavy chain of lebrikizumab	
QVTLRSGPALVKPTQTLTLTCTVSGFSL SAYSVN EWIRQPPGKAL WLAMIWGDGKIVY NSAL KSRLTISKDTSKNQVVLMTNMDPVDATATYYCAGDGY PPYAMD NWGQGLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPS SSLGKTYTCNVDHKPSNTKVDKRVESK	
ABR1:	FSL SAYSVN (27-35)
ABR2:	WLAMIWGDG KIVY (47-59)
ABR3:	GDGY PPYAMD N (97-107)
Paratope of light chain of lebrikizumab	
DIVMTQSPDLSVSLGERATINCRAS KSVDSYGNSFMH WYQQKPGQPPK LLIYLASNLES GVP DRFSGSGSGTDFTLTISSLQAEDVAVYYC QQNNEDPR TFGGGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC	
ABR1:	KSVDSYGNS FMH (27-38)
ABR2:	LLIY LASNLES (50-60)
ABR3:	QQ NNEDPR (93-100)

Figure 4. Analysis of paratope regions of IL-13 – lebrikizumab complex. Complementary determining regions are highlighted in different colors.

Table 2. Predicted epitope sequence present on Interleukin-13 with their propensity score.

Rank	Sequence	Start position	Score
1	ELIEELVNIT	11	0.79
2	ITQNQKAPLC	19	0.74
3	APLCNGSMVW	25	0.71
4	PVPPSTALRE	2	0.68
5	NVSGCSAIEK	52	0.66
6	GMYYCAALES	41	0.64
7	ELVNITQNQK	15	0.62
8	SINLTAGMYC	35	0.60
9	TQRMLSGFCP	62	0.57

stability and favourability of each mutation along with a change in free energy. Based on these three criteria out of proposed 8133 mutations, 1199 mutations were filtered that were having the positive $\Delta\Delta G$ score. Out of these 1199 mutations, 40 mutations were selected (Supplementary Table 1) that involve only interface residues of lebrikizumab. We didn't select the unstable and unfavorable mutations for further analysis because we wanted to discover the mutations which enhance binding affinity with a stable structure of the lebrikizumab. From all the criteria we have estimated that the change in free energy after mutation indicates N53V;

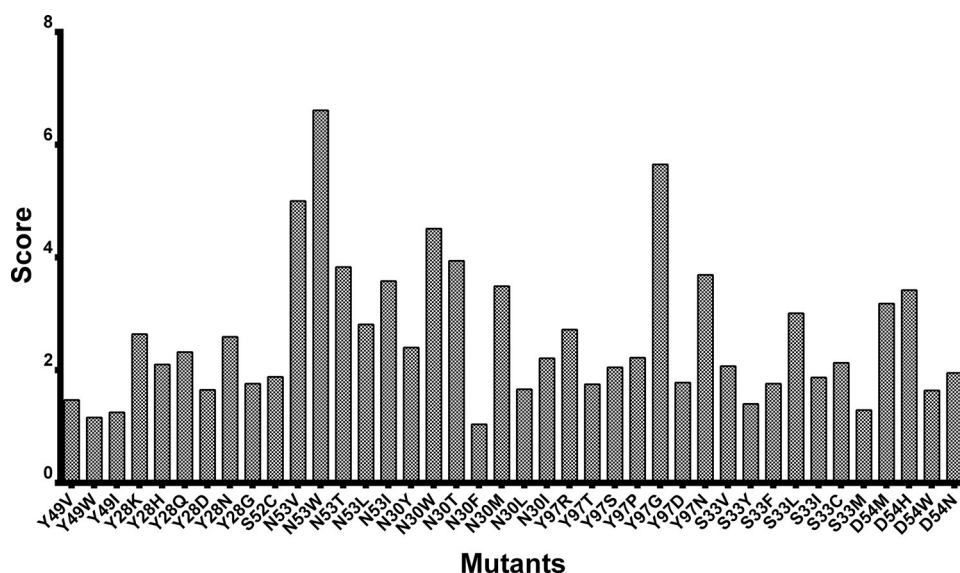


Figure 5. Plot representing the most stable and favorable mutations in lebrikizumab on the basis of $\Delta\Delta G$ derived from the CUPSAT web server.

Table 3. Z-dock scores of top 40 stable and favorable single mutations in lebrikizumab.

S No.	Chain Id	Residue	Mutation	Z-Score
1	L	TYR49	VAL	1658.463
2	L	TYR49	TRP	1753.97
3	L	TYR49	ILE	1675.007
4	L	TYR28	LYS	1628.658
5	L	TYR28	HIS	1618.449
6	L	TYR28	GLN	1632.536
7	L	TYR28	ASP	1561.653
8	L	TYR28	ASN	1600.408
9	L	TYR28	ARG	1609.325
10	L	SER52	CYS	1719.25
11	L	ASN53	VAL	1743.94
12	L	ASN53	TRP	1975.983
13	L	ASN53	THR	1772.457
14	L	ASN53	LEU	1777.702
15	L	ASN53	ILE	1776.648
16	L	ASN30	TYR	1909.209
17	L	ASN30	TRP	1979.3
18	L	ASN30	THR	1719.365
19	L	ASN30	PHE	1892.003
20	L	ASN30	MET	1799.056
21	L	ASN30	LEU	1794.166
22	L	ASN30	ILE	1768.346
23	L	ASN30	ARG	1799.55
24	H	TYR97	THR	1495.046
25	H	TYR97	SER	1519.350
26	H	TYR97	PRO	1541.814
27	H	TYR97	GLY	1650.832
28	H	TYR97	ASP	1391.849
29	H	TYR97	ASN	1464.475
30	H	SER33	VAL	1720.165
31	H	SER33	TYR	1772.121
32	H	SER33	PHE	1854.837
33	H	SER33	LEU	1717.040
34	H	SER33	ILE	1728.014
35	H	SER33	CYS	1719.895
36	H	SER30	MET	1719.895
37	H	ASP54	MET	1918.175
38	H	ASP54	HIS	1787.266
39	H	ASP54	TRP	1949.193
40	H	ASP54	ASN	1773.483

N53W; N53T; N53I; N30W; N30T; N30M (the first alphabet indicates residue of wild type; Number indicates the position of residue; the second alphabet indicates mutation) in the light chain and Y97G; Y97N; S33L; S33F; D54M; D54H in the

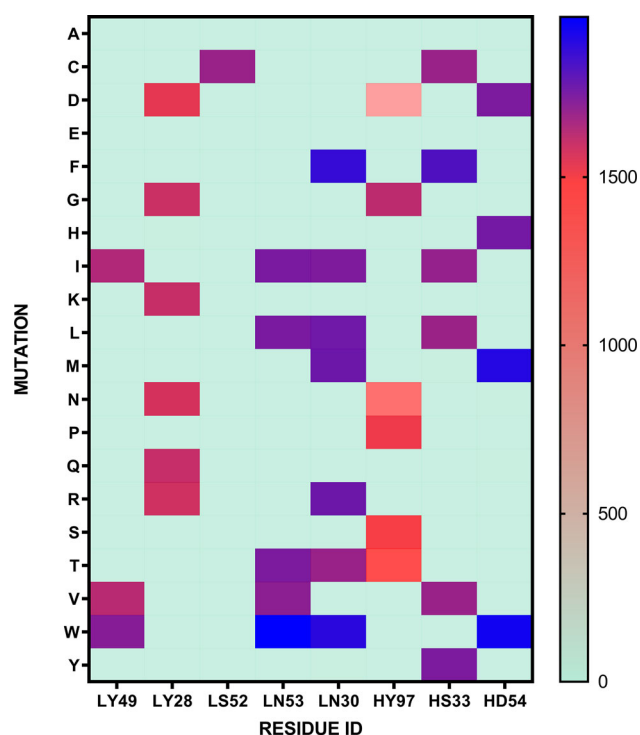


Figure 6. Heatmap of mutations representing the Z-score of different single mutants of lebrikizumab. The blue color is indicating the mutations that increases the binding affinity.

heavy chain were the most stable and favorable mutations (Figure 5). With the identification of these 40 mutations, a single mutant library was created by using PyMOL.

Generation of the multi-mutant library of lebrikizumab

To identify those residues that account for the increase in the binding affinity of lebrikizumab towards the IL-13, we subjected the 40 selected mutants from CUPSAT (single mutant library) to Z-Dock web server. In order to select the mutations with an enhanced binding affinity for creating the

multi-mutant library, the derived scores (Table 3) of these 40 single mutants were compared with the standard score of wild type lebrikizumab (Z score: 1719.26) (Figure 6). The mutations above the Cut-off of 1800 score (D54M; D54W; S33F) in heavy chain and (N30F; N30W; N30Y; N53W) in the light chain were further selected, and a multi-mutant library of 40 diverse combinations including doublet, triplet, and quadrat mutation was generated (Supplementary Table 2).

Table 4. Z-dock score based binding affinity prediction for the sorted forty mutant combinations.

S NO.	Mutations				Z Scores
	Heavy-54D	Heavy-33S	Light-30N	Light-53N	
1	–	–	F	W	2155.080
2	–	F	F	–	2033.971
3	M	F	–	–	1898.611
4	M	–	W	–	2095.879
5	M	–	F	–	2012.439
6	–	F	–	W	2035.541
7	W	F	–	–	2027.465
8	W	–	–	W	2215.569
9	W	–	F	–	2128.189
10	–	–	W	W	2242.431
11	–	F	W	–	1857.518
12	M	–	W	–	2095.879
13	W	–	W	–	2215.569
14	–	–	Y	W	2172.289
15	–	F	Y	–	2014.152
16	M	–	Y	–	2010.874
17	W	–	Y	–	2145.37
18	–	F	F	W	2214.635
19	M	F	F	–	2067.006
20	M	–	F	W	2211.133
21	M	F	–	W	2091.733
22	W	F	F	–	2217.196
23	W	–	F	W	2391.208
24	W	F	–	W	2271.799
25	–	F	W	W	2301.989
26	M	F	W	–	1922.408
27	M	–	W	W	2298.568
28	W	F	W	–	2287.37
29	W	–	W	W	2478.635
30	–	F	Y	W	2231.849
31	M	F	Y	–	2085.08
32	M	–	Y	W	2228.348
33	W	F	Y	–	2235.263
34	W	–	Y	W	2408.289
35	M	F	F	W	2270.691
36	W	F	F	W	2270.693
37	M	F	W	W	2270.692
38	W	F	W	W	2539.108
39	M	F	Y	W	2287.906
40	W	F	Y	W	2467.972

Affinity assessment of potential neutralizing mutant monoclonal antibodies

After generating the multi-mutant library of 40 diverse mutant combinations, docking was reperformed with Z-dock (Table 4) and filtered the best ten combinations (Table 5) based on high Z scores among all. These ten antibodies further screened by PatchDock and ClusPro Docking and compared with scores of a native complex of lebrikizumab and IL-13 (Z-dock score: 1719.26, PatchDock score: 9590, ClusPro minimum energy: –452 and interface score: –291.4). The greater Z-dock and PatchDock scores whereas, lower ClusPro energy and interface score indicate potential mAb with better affinity and specificity for the target cytokine. The mutant mAb No. 5 (mAb-5) shows the highest affinity for the IL-13 with its respective scores (Z-Dock score: 2539, PatchDock score: 15344; ClusPro minimum energy: –505.3 and interface score: –315.5). The second best theoretical mAb was mAb-10 with scores (Z-dock score: 2408, PatchDock score: 10292, ClusPro minimum energy: –499.6 and interface score: –345.7). These scores indicate that mAb-5 and mAb-10 could be used as potential antibodies after *in-vitro* and *in-vivo* validation to neutralize the IL-13 with greater efficacy than lebrikizumab.

MD Simulation to verify stable interaction

The conformation of having a stable complex between mAb5-IL13 and mAb10-IL13 was determined by using Gromacs standalone software and compared with the wild-type lebrikizumab-IL-13 complex. The whole simulation was performed for the duration of 20ns, and the trajectory was analyzed to make an RMSD and RMSF plot. The former plot represents that lebrikizumab-IL-13, mAb5-IL13, and mAb10-IL13 complex having an average RMSD value of 0.2nm. All these RMSD values are very less and comparable with each other, representing the stable nature of the complex (Figure 7(A, C, E)). Secondly, the RMSF plot reflects a similar value of approx. 0.12nm, again representing the stable complex formation (Figure 7(B, D, F)).

Evaluation of H-bonding and hydrophobic interaction of mutated residues

Using Ligplot + the difference in H-bonding and hydrophobic interaction of wild type residues (Figure 8) and the mutated

Table 5. Docking scores of top 10 theoretical antibodies and lebrikizumab with highlighted best two antibodies.

S No.	Mutations	Z-Score	PatchDock	ClusPro	
				Minimum energy	Central energy
1	HD54W; HS33F; LN30F	2217.196	9606	–468.1	–328.7
2	HD54W; HS33F; LN30W	2287.370	9848	–497.5	–366.5
3	HD54W; HS33F; LN53W	2271.799	9860	–471.9	–353
4	HD54W; LN30F; LN53W	2391.208	9914	–474.1	–334.3
5	HD54W; HS33F; LN30W; LN53W	2539.108	15344	–505.3	–315.3
6	HD54W; HS33F; LN30Y	2235.263	9840	–489.1	–325.1
7	HD54W; HS33F; LN30Y; LN53W	2467.972	9636	–494.7	–338
8	HD54W; LN30W	2215.569	9646	–485.6	–322.1
9	HD54W; LN30W; LN53W	2478.635	9914	–483.3	–335.4
10	HD54W; LN30Y; LN53W	2408.417	10292	–499.6	–345.7
11	WILD TYPE	1719.26	9590	–452	–291.4

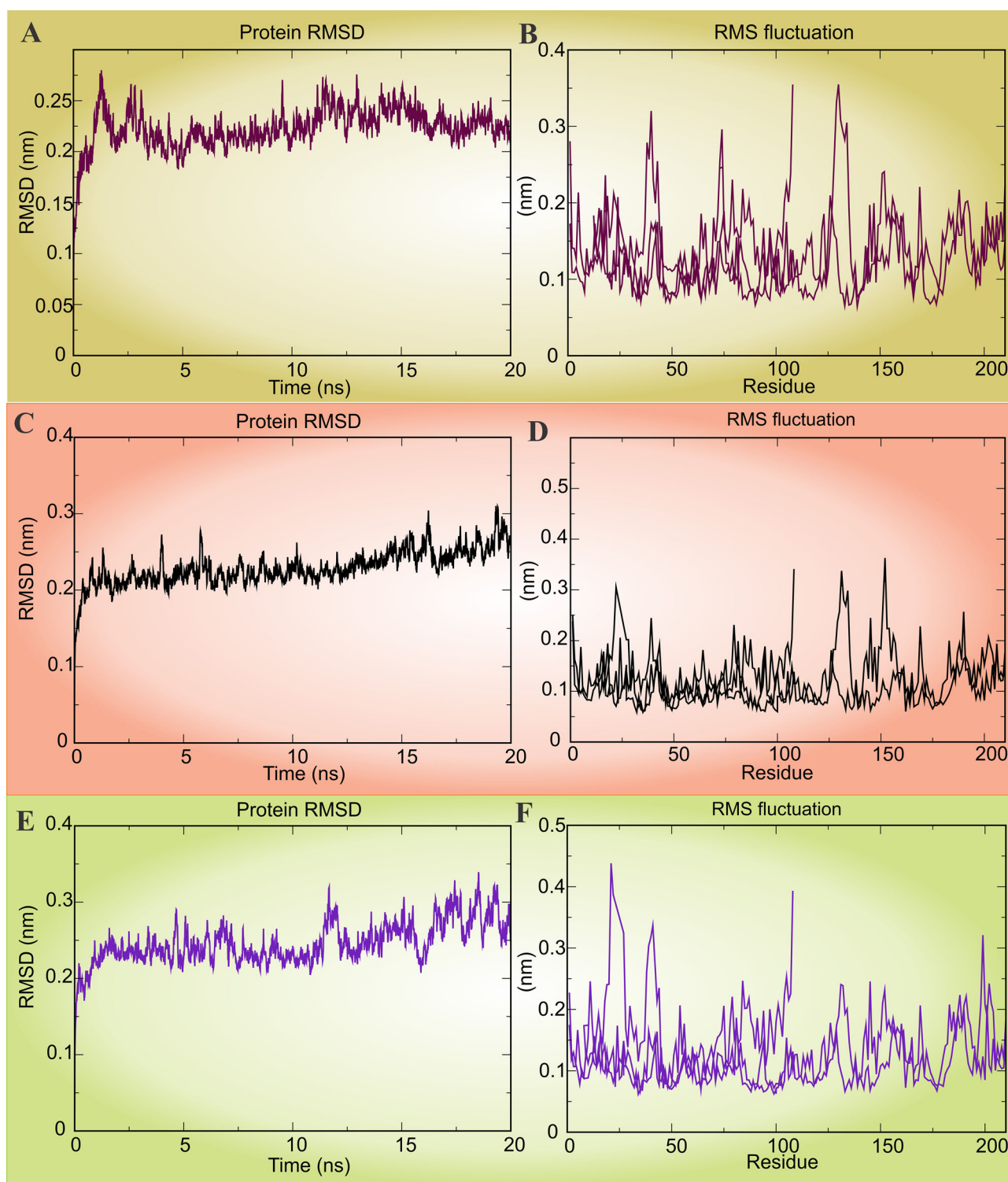


Figure 7. RMSD and RMSF plot of Lebrikizumab (maroon); mAb5 (black); mAb10 (indigo).

residue was determined, which accounts for the change in the affinity of mAb towards the IL-13. Mutation of S33 of lebrikizumab with phenylalanine leads to the formation of four more hydrophobic interactions in mAb-5 (Figure 9). In mAb 10 (Figure 10), it was observed that change of N30 and N53 of light chain with tyrosine and tryptophan, respectively, resulted in twelve more interactions compared to three hydrophobic bonds of native lebrikizumab. The replacement

of paratope residues with aromatic amino acid facilitates more hydrophobic interaction in mAb-5 and mAb-10.

Allergenicity, toxicity and physiochemical properties determination of mutants

Using the AllerTOP and ToxinPred server, it was found that there was no change in the toxicity and allergenicity of

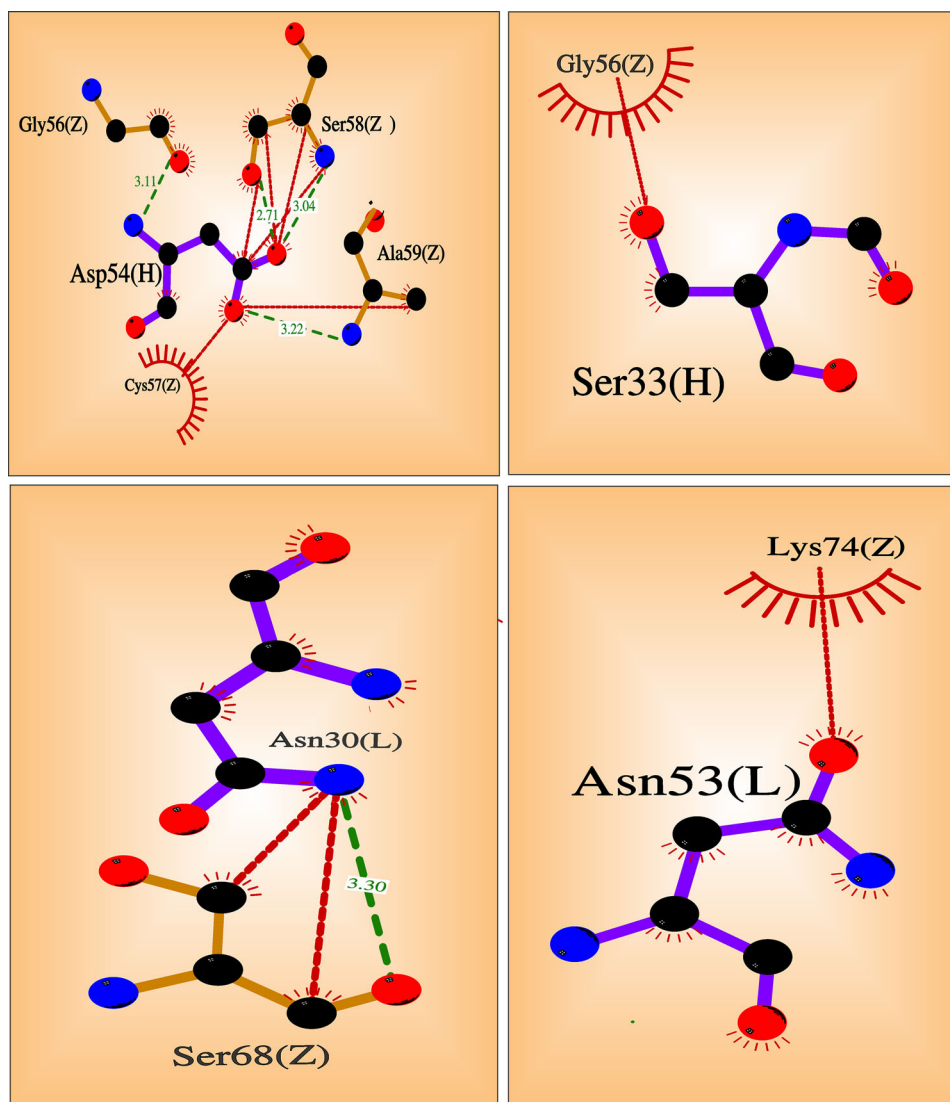


Figure 8. LigPlot diagrams indicating hydrophobic interaction and H-bonding of native lebrizumab residues (N54, S33) of the heavy chain and (N30, N53) of light chain with IL-13 (Z chain). The dotted red line represents hydrophobic interaction, and the green line indicates hydrogen bond.

lebrizumab after mutations. All three mAbs (lebrizumab, mAb5, and mAb10) were non-toxic (Supplementary Tables 3 and 4) and non-allergic in nature. Therefore, this mutated lebrizumab can be designed for better affinity and efficacy against IL-13 without any toxicity and allergy. The physicochemical evaluation reveals that half-life of all three that is a wild type and mAb-5 and mAb-10 was 0.8 h in mammalian reticulocyte. The instability index (wild type: 34.68; mAb5: 37.37 and mAb10: 37.92) indicates all three are stable protein. The aliphatic index was also the same (75.30), whereas the GRAVY score (Grand Average of Hydropathicity) was different (wild type: -0.216 ; mAb5: -0.198 ; mAb10: -0.188) which indicate the negative score indicates that the wild type and mutated mAb are hydrophilic in nature.

Discussion

Mutations have both advantages and disadvantages, but mutations generated in the mAb to improve the binding efficacy with inflammatory molecules may be beneficial at the therapeutic level. The selection of these advantageous

mutations is a significant challenge of immunotherapeutics and needs scientifically systematic efforts and expertise in bioinformatics. Here we have tried to normalize the algorithm used to detect the precise amino acid position whose mutation leads to generate an efficient antibody against IL-13 inflammatory cytokines leading to various diseases. This study implies the same algorithm to find out the position-specific mutations in the existing mAb, namely lebrizumab, with the possibility to enhance its binding affinity for IL-13 (Rael & Lockey, 2011). Currently, there is a total of five monoclonal antibodies under clinical trials, namely IMA-638, CNT05825, IMA-026, Tralokinumab (CAT-354), and Lebrizumab. The IMA-638 has shown better response towards allergy than IMA026, but there was no improvement in other symptoms when administered with IMA-638 (Gauvreau et al. 2011; O'Byrne et al., 2009). Tralokinumab is a humanized IgG4 mAb that can reduce the effect of IL-13. It is associated with many adverse effects like nasal mucus hypersecretion (sinusitis), headache, and nasopharyngitis (Catley et al., 2011; Oh et al., 2010). Among all five clinically verified monoclonal antibodies, lebrizumab has shown

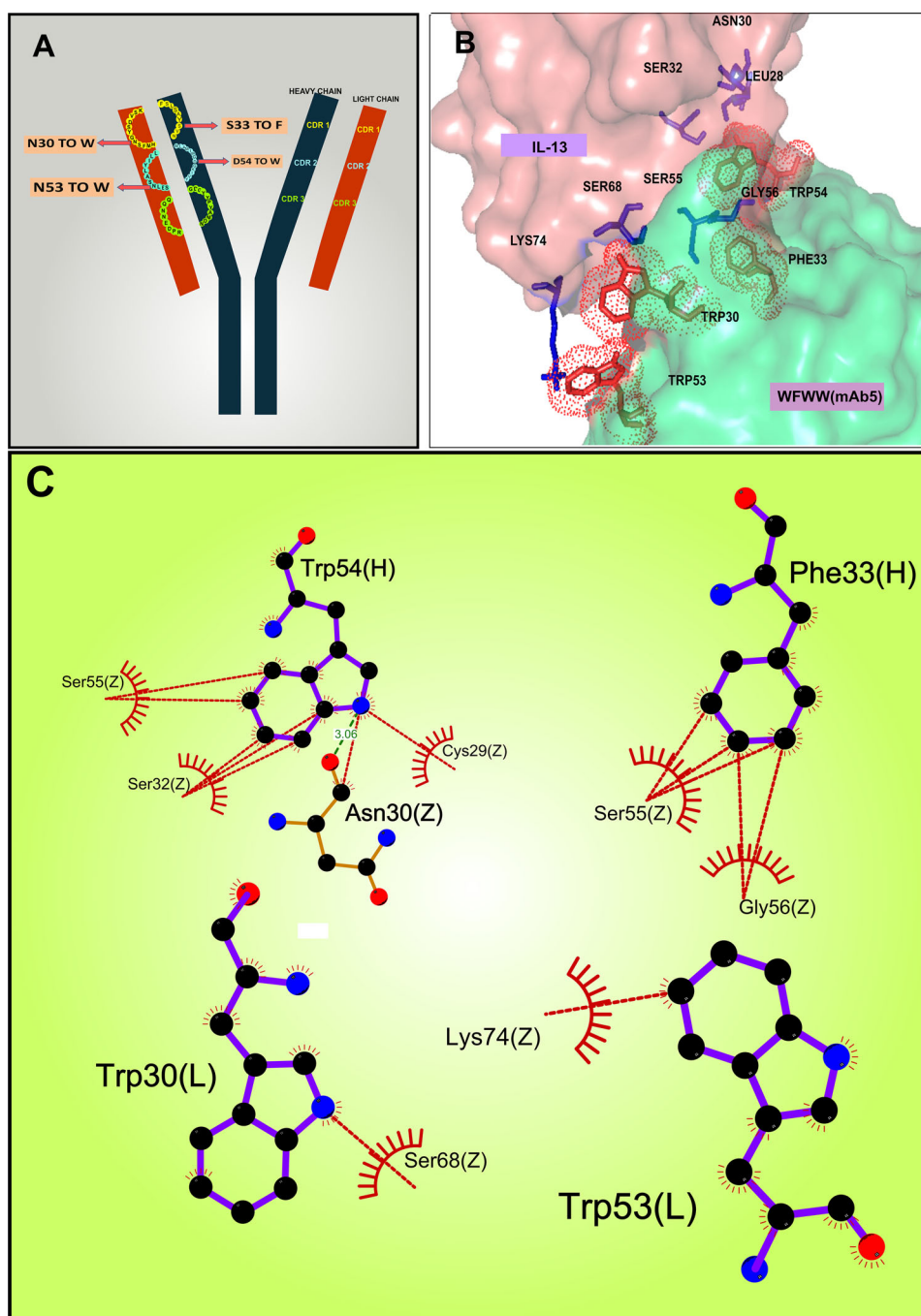


Figure 9. Interaction between mutated mAb (mAb5) and IL-13. Mutations in the mAb5 and its interaction between mutated residue and IL-13 residues with their LigPlot analysis of hydrophobic interaction and H-bonding.

lower dissociation constant (< 10 pM) (Ultsch et al., 2013) of 14–164 pM against IL-13, indicating high binding affinity (Kasaian et al. 2008; May et al., 2012). Lebrikizumab is capable of improvising the lung function in asthmatic patients in high periostin level, but it was unable to give expected results in secondary outcomes of clinical trials (Corren et al., 2011), therefore, needs further modification to achieve the desired goal. Here, lebrikizumab was selected and modified by a mutational approach using a rigorous immunoinformatics algorithm to improve its affinity and specificity for IL-13. Interestingly, the resulting mutant antibodies have shown a significant affinity towards IL-13 and made a stable complex as determined by an MD simulation study. From the

beginning, we tried to introduce a simple mutational approach to improve the binding affinity of lebrikizumab for its natural antigen IL-12. But as per literature reported elsewhere, the affinity obstacles of the lebrikizumab towards IL-13 can be improved by mutational approaches by utilizing Perl script, which is a high-level dynamic programming language (Saba Khan et al., 2018; Verma et al., 2016). When we apply this language to create mutations in any biomolecule, it creates very complex data of the mutant library. This complex data cannot be analyzed without any prior knowledge of this language. Therefore, here we have tried to reduce the complexity of the data by standardizing user-friendly protocol and did the same by using the CUPSAT web server and

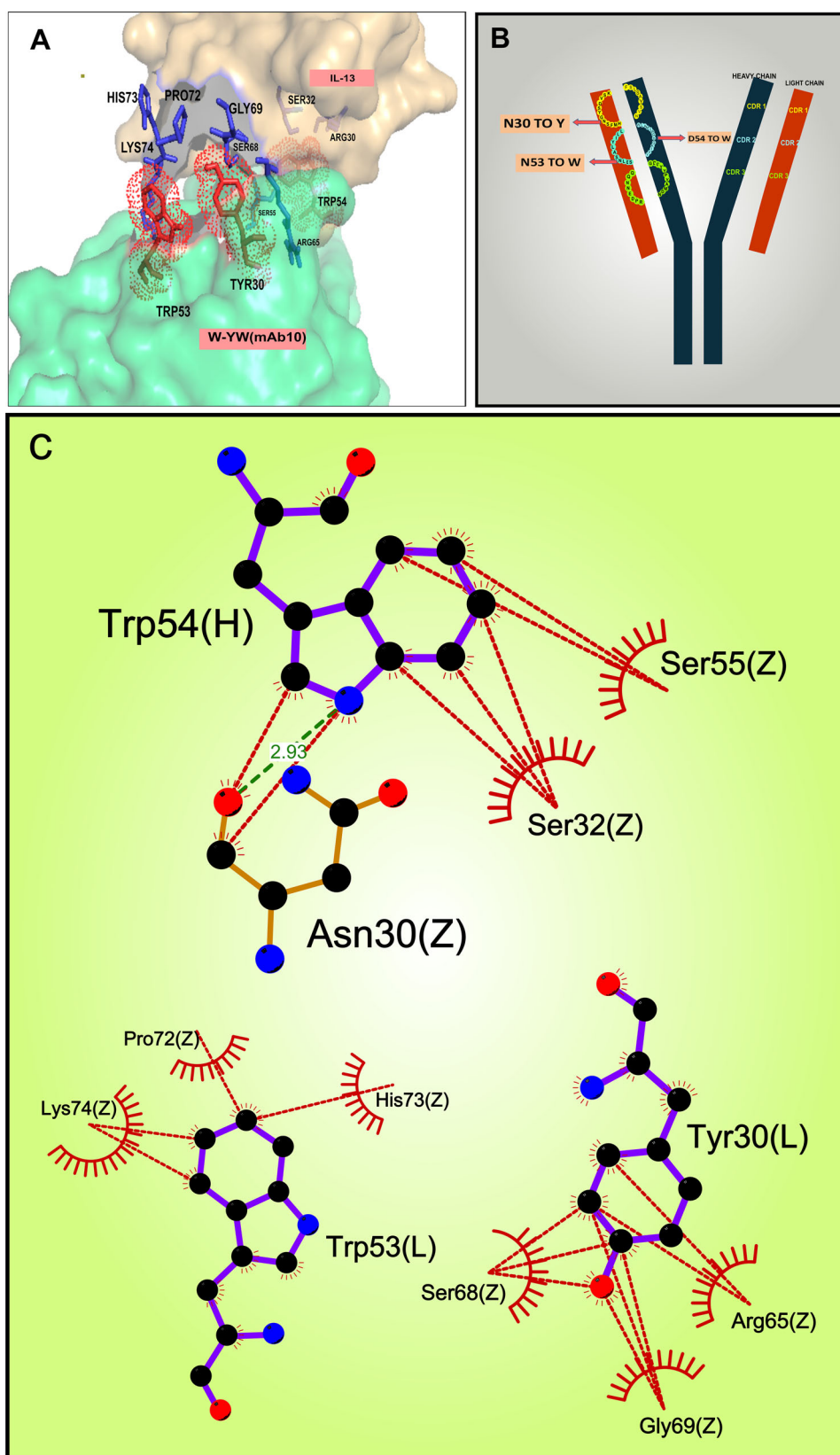


Figure 10. Interaction between mutated mAb (mAb10) and IL-13. Mutations in the mAb10 and its interaction between mutated residue and IL-13 residues with their LigPlot analysis of hydrophobic interaction and H-bonding.

created a mutant library of lebrikizumab. We found that out of all mutations, only four-point mutations (HD54W, HS33F, LN30W/Y, and LN53W) were responsible for increasing the affinity and stability of the lebrikizumab towards the IL-13.

The interaction and binding affinity of mutated lebrikizumab with IL-13 were validated by three docking servers Z-dock, PatchDock, and ClusPro, as reported elsewhere (Kozakov et al., 2017; Leone et al., 2012; Pierce et al., 2011). The

docking scores and free energy of all mutants of lebrikizumab from these servers were compared with the wild type of lebrikizumab. Finally, out of all mutations, we have selected only two combination mutant antibodies (WFWW (mAb5) and W-YW (mAb10) of lebrikizumab, which was showing good affinity with the IL-13 based on docking analysis. We also analyzed that these two mutants are non-allergic and nontoxic using AllerTOPv 2.0 and ToxinPred web servers, respectively.

Moreover, this study also performed better on the parameters reported in the previous study, which describes that the binding residues of the paratope region are mostly occupied by aromatic amino acid residues that interact with the epitope regions of the antigen (Kringelum et al., 2013; Peng et al., 2014; Ramaraj et al., 2012). In our result, we showed that the selected positions for mutations that were replaced by aromatic amino acids, only those mutations, were showing good affinity with IL-13. This study opens new doors to finding beneficial mutations which can improve the therapeutics against different chronic diseases. This indicates that the final mutant antibodies (mutated antibody –5 and –10, Table 2) may have the capability to significantly reduce the asthmatic symptoms and improve hyperactive response.

Conclusion

This study improves the affinity of the existing mAb lebrikizumab against IL-13 by creating point mutations. We found that multiple point mutant combinations (mAb –5 and –10 Table 2) were showing better affinity with IL-13 as compared to wild type. The interaction of IL-13 and mutated lebrikizumab were also analyzed by three docking servers for a reliable result. The residues which were responsible for increasing the affinity towards IL-13 were only aromatic residues. The designed antibodies were also validated at the microscopic level for their type of interactions, and their thermodynamics were performed using MD simulations. The designed antibodies were not showing any allergenicity and toxicity as the effect of mutations and can go for the *in vitro* and *in vivo* evaluation.

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Author contribution

Protocol designed by VS, PC, MK, RKP and VKP. Methodology performed by VS, PC, RO, VSA. The manuscript was written by RKP, PC, VS, and VKP.

Disclosure statement

The authors have declared no competing interest.

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