In Silico Design of a Novel Chimeric DT380-CD19 Recombinant Immunotoxin for Targeting Human B-Cell Lymphoma

Davood Mohammadbagher [1, 2], Mojtaba Noofeli [2], Mohammad Tabatabaei [1]*, Majid Esmaelizad [2], Azadeh Yektaseresht [1],

[1] Department of Pathobiology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran.

[2] Research & Development Department, Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

* Corresponding authors: Mohammad Tabatabaei, mtabatabaei2003@yahoo.co.uk,

Abstract

Introduction: Cancer remains a major global health challenge, with high morbidity and mortality rates. Traditional cancer therapies often cause adverse side effects due to their non-specific function, which has led to the exploration of targeted therapies, such as immunotoxins. Despite their high potential, these therapies face hurdles, including immunogenicity, side effects and low effectiveness. This research aims to explore a novel immunotoxin with reduced immunogenicity, improved safety and efficacy.

Materials and Methods: Through a comprehensive bioinformatics analysis, this study primarily evaluates the immunogenicity and docking interactions of a novel recombinant immunotoxin. The designed immunotoxin is created by combining the shortest truncated form of diphtheria toxin (consisting of 380 amino acids) with a single-chain variable fragment monoclonal antibody synthesized in an *E. coli* expression system for specific targeting of the human CD19 antigen.

Results: In silico analyses show that the newly designed immunotoxin has acceptable immunogenicity and an improved docking profile compared to existing versions of

immunotherapeutics. This suggests the potential of DT380-CD19 as a more effective and safer option for targeted therapy of CD19+ B-cell malignancies.

Conclusion: The findings declare DT380-CD19 immunotoxin potential as a more effective and safer option for B-cell malignancy targeted therapy than former therapeutics. Future research must underscore the necessity of integrating both computational and experimental approaches to refine immunogenicity without compromising the effectiveness of immunotoxin therapy, ultimately striving to improve patient outcomes in cancer treatment.

Key words: CD19+ B-cell malignancy; immunogenicity; immunotoxin; targeted therapy

1. Introduction:

Cancer, with 20 million deaths per year, remains a leading global cause of death. $CD19^+$ B-cell malignancies are significant health concerns worldwide, accounting for about 30% of all blood cancers according to the World Health Organization. In the United States, approximately 200,000 new cases are reported each year [1]. Conventional cancer treatments such as surgery, chemotherapy, radiotherapy, and stem cell transplantation often come with nonspecific toxicities and severe side effects, harming healthy cells along with cancerous ones [2]. These challenges have prompted the development of targeted therapies that offer improved outcomes by distinguishing between healthy and cancerous cells [3].

Targeting CD19+ B-cell lineage antigens is advantageous because this antigen remains present on malignant cells in many B-cell malignancies, enabling selective killing. The use of naked anti-CD19 antibody targeted therapy has shown promising results and revolutionized the treatment of CD19+ B-cell malignancies. However, it did not meet all the requirements. Consequently, Blinatumomab, an FDA-approved bi-specific T cell engager (BiTE) antibody targeting CD19 and CD3, was developed to enhance efficacy in treating relapsed or refractory B-cell malignancies and autoimmune diseases [4,5].

Targeted therapy has garnered considerable attention in recent decades, particularly with the development of immunotoxins (ITs) that merge the targeting capabilities of monoclonal antibodies with the cytotoxic effects of toxins to boost specificity and potency while minimizing size. The initial FDA-approved DTx-based ITs, DT389-IL2 (OntakTM) and DT388-IL3 (Elzonris), consist of truncated diphtheria toxin (DTx) fused with human interleukin 2 and 3 [6].

In addition to efficacy, the immunogenicity of anticancer drugs is a significant concern [7]. The aim of this study was to develop a novel IT to enhance the treatment efficacy for B-cell malignancies by minimizing immunogenicity and off-target binding.

2. Materials and Methods:

2.1. Sequence Designing

Fragments of DT380 and the human anti-CD19 antibody were used in the development of fusion IT. The sequence for DT380 was obtained from the native DTx (UniProt ID: P00588), Ontak (DrugBank accession no. DB00004), and Elzonris (DrugBank accession no. DB14731) ITs [8-10]. The native DTx sequence consists of a signal peptide (amino acids 1-32), fragment A (C domain, amino acids 33-225), and fragment B (T and B domains, amino acids 226-567), totaling 567 amino acids. In the new fusion IT, the R binding domain was replaced with the human anti-CD19 scFv (VH & VL) antibody (Blincyto, DrugBank accession no. 09052) as the binding domain at the C-terminal end of the IT [11]. To maintain the structural and functional properties of the novel IT, the variable heavy (VH) and light (VL) chains of the anti-CD19 scFv antibody were connected to each other and to DTx using (G4S)3 and G4S hydrophobic flexible linkers, respectively.

Cleavage sites in the pET-28a(+) plasmid expression vector (Novagen) were utilized to insert NcoI-XhoI restriction enzyme sites at the 5′ and 3′ ends of the DT380-CD19 sequences. To improve cloning efficiency and ensure high-level expression of the chimeric IT, the *E. coli* BL21 (DE3) Rosetta strain (Novagen) was used as the host. [12]. Chloramphenicol and kanamycin were utilized as selectable antibiotics for the Rosetta strain and the pET28a(+) expression vector, respectively [13]. The constructs were chemically synthesized by Shine-Gene of Molecular Biotech Co. (Shanghai, China) and then inserted into the pET28a(+) expression vector.

The DT390-CD19 sequence includes DT390 and FMC63 (anti-CD19 scFv), Zynlonta (anti-CD19 mAb drug conjugate), Tafasitamab (anti-CD19 mAb), 6AL5 (protein-protein complex of B43Fab antibody with CD19 (N138Q mutant) antigen), SJ25C1 (Fab antibody complex with CD19 antigen), and human CD19 antigen (UniProt P15391). These components were utilized in the study and can be found in the supplementary information section [14-19].

2.2. Messenger RNA Secondary Structure Prediction

The Vienna RNAfold web server, version 2.0, was employed to predict the secondary structure of messenger RNA (mRNA) and analyze RNA sequences. (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) [20].

2.3. Primary Protein Structure Estimation

The physicochemical properties of the protein were determined using the ExPASy ProtParam web server (http://us.expasy.org/tools/protparam.html) [21].

2.4. Secondary Protein Structure Evaluation

The PROTEUS Structure Prediction Server 2.0 web server (http://www.proteus2.ca/proteus2/) was used to evaluate the secondary protein structure [22].

2.5.Tertiary Protein Structure Analysis

The 3D structure models were analyzed using Swiss homology and I-TASSER non-homology de novo models on online servers (https://swissmodel.expasy.org/interactive) and (https://zhanggroup.org/I-TASSER/) [23,24]. The quality of the protein 3D models was verified using the UCLA-DOE LAB-SAVES v6.0 online server, (https://saves.mbi.ucla.edu/) [25].

2.6. Solubility Assessment

The protein's solubility was assessed using the Protein-Sol online server (https://protein-sol.manchester.ac.uk/) [26].

2.7. Toxicity Determination

Toxicity was evaluated by identifying toxic structural regions using the ToxinPred web server. (https://webs.iiitd.edu.in/raghava/toxinpred2/) [27].

2.8. Antigenicity &Allergenicity Prevision

Antigenic sites were predicted using the VaxiJen 2.0 web server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), and their allergenicity was assessed by comparing them to known IgE epitopes using the Algpred web server (https://webs.iiitd.edu.in/raghava/algpred/submission.html) [28,29].

2.9. B & T- lymphocyte Epitopes Determination

Linear (continuous) and conformational (discontinuous) antigenic epitopes were identified using the SVMTriP (http://sysbio.unl.edu/SVMTriP/) and IEBD-Ellipro (http://tools.iedb.org/ellipro/) web servers [30,31].

The T-cell epitopes were determined by analyzing the major histocompatibility complex (MHC) I and II binding regions using the ProPred-I (https://webs.iiitd.edu.in/raghava/propred1/index.html) and IEDB-MHCII (http://tools.iedb.org/mhcii/) web servers, respectively [32,33].

2.10. Ligand-receptor Docking

To investigate the positioning and interactions of ligands with the human CD19 receptor, protein docking and binding affinity analyses were performed using HawKDocK (http://cadd.zju.edu.cn/hawkdock/), HADDOCK 2.4 (https://rascar.science.uu.nl/haddock2.4/), PRODIGY (https://rascar.science.uu.nl/prodigy/), and PPA-Pred2 (https://www.iitm.ac.in/bioinfo/PPA_Pred/) web servers, respectively [34-37].

3. Results:

3.1. Sequence Designing

The schematic diagram of the chimeric IT comprising C, T, and scFv anti-CD19 domains is depicted in Figure 1.

Fig. 1. Schematic representation of DT380-CD19

3.2. Codon optimization

Over 200 factors influence gene expression, and they need to be carefully screened and optimized to enhance the likelihood of obtaining a functional and active protein.

3.2.1. Guanine-cytosine content % (GC)

The GC content of DT380-CD19 was adjusted from 56.78% to 53.58%, bringing it closer to the *E. coli* genome range of 50.4-50.8%. This adjustment resulted in higher expression levels [38].

3.2.2. Codon adaptation index (CAI)

The CAI measures the similarity between a gene's codon usage and the preferred codon usage of the target organism. The values range from 0 to 1, with higher scores indicating better adaptation to codon usage and increased expression levels [16]. DT380-CD19 achieved a CAI of 1[38].

3.2.3. Transfer RNA Adaptation Index (tAI)

The Translational Activity Index (TAI) assesses translational efficiency based on tRNA levels and the effectiveness of codon-anticodon interactions. DT380-CD19 was assigned a TAI score of 0.24 [38].

3.2.4. Effective number of codon pairs (ENCP)

The ENCP assigns a numerical value to indicate bias in codon pair usage, ranging from 20 to 62. A higher value suggests uniform usage of all synonymous codons without bias. DT380-CD19 had an ENCP value of 20 [38].

3.3. Messenger RNA Secondary Structure Prediction

Predicting the secondary structure of mRNA is essential for understanding structure-function relationships, enhancing stability, and optimizing translation efficiency [20].

3.3.1. Minimum free energy (MFE)

The MFE represents the free energy value of the most stable RNA structure in Kcal/mol, with a more negative value indicating a more stable structure. Lower values indicate more stable structures. The MFE value of the DT380-CD19 RNA was - 623.10 Kcal/mol [20].

3.3.2. Free energy of thermodynamic ensemble (FET)

The free energy of the structure (FET) is calculated using the partition function algorithm to generate a dot-plot matrix that shows base-pair probabilities. Lower FET values indicate more stable structures. The FET value for the DT380-CD19 RNA was -654.93 [20].

3.3.3. Centroid minimum free energy (CMFE)

The CMFE predicts RNA secondary structures by considering the minimum base-pair distance to all other structures in the Boltzmann ensemble. Lower values indicate greater stability. The CMFE value for the DT380-antiCD19 RNA was - 498.85 [20].

3.4. Primary Protein Structure Estimation

The physicochemical properties of DT380-CD19 can provide insights into its behavior. These properties include a molecular weight of 68.14 kD, an isoelectric point of 5.09, a negative surface charge/positive surface charge ratio of 72/54, a half-life of 30 hours in mammalian reticulocytes, >20 hours in yeast, and >10 hours in *E. coli*, an instability index of 39.51, an aliphatic index of 73.38, and a GRAVY score of -0.368. Additional data can be found in Table 1 of the supplementary information [21].

3.5. Secondary Protein structure evaluation

The secondary structure analysis of DT380-CD19 revealed 26% alpha helix (164 residues), 27% beta sheet (172 residues), 47% turn or random coil (298 residues), and 0% transmembrane or signal peptide (0 residues) [22].

3.6. Protein Tertiary Structure Analysis

The SWISS and I-TASSER online modeling servers were utilized to create 3D models of IT, anti-CD19, and CD19 fragments. The I-TASSER modeling predictions were selected for their superior sequence matching. The results can be found in Table 2, and the top 3D SWISS model results are presented in Table 3 of the supplementary information [23,24].

3.6.1. Confidence score

The C-score, which measures model accuracy and quality, typically ranges from -5 to 2. A higher score indicates better quality. The DT380-CD19 C-score was -1.63 [24].

3.6.2. TM-alignment score

The TM value is a measure of similarity between a predicted model and a known structure in the PDB library, with a range from 0 to 1. Higher TM values indicate better model quality. The TM value for DT380-CD19 was 0.52 ± 0.15 [24].

3.6.3. Root mean square atomic deviations

The RMSD is a metric that measures the average distance between residue pairs in the predicted model and the native structure. A lower RMSD value indicates a better alignment. The RMSD score of DT380-CD19 was 11.8 ± 4.5 Å [24].

Figure 2 shows the most optimal 3D images of DT380-CD19 and its corresponding fragments.

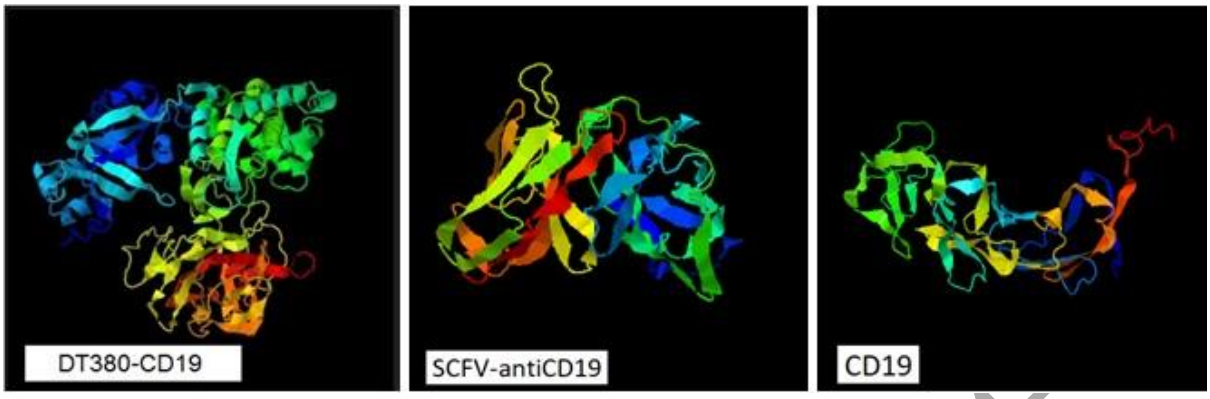

Fig. 2. Top 3D images

3.7. 3D structure verification

The verification results of the top 3D models are presented in Table 4 of the supplementary information.

3.7.1. VERIFY3D

The 3D verification tool assesses the alignment between a 3D atomic model and its corresponding amino acid sequences (1D). A pass is considered successful if at least 80% of the amino acids have a score of ≥ 0.1 in the 3D/1D profile. The VERIFY3D score for DT380-CD19 was 81.39% [25].

3.7.2. ERRAT

The ERRAT tool evaluates the quality of crystallographic model building and refinement. A higher ERRAT value indicates better structural quality. The ERRAT score for DT380-CD19 was 7.237 [25].

3.7.3. PROCHECK

PROCHECK analyzes the stereo chemical quality of protein structures by examining residue-by-residue and overall structure geometry. The PROCHECK scores for DT380-CD19 were 70.7 % (Favored), 25.2% (Allowed), and 4.1 % (Disallowed) [25].

3.8. Solubility assessment

The average solubility score of proteins in E. coli is 0.450. Scores above this threshold indicate solubility. The predicted solubility scores for DT380-CD19 and DT390-CD19 were 0.415 and 0.381, respectively [26].

3.9. Toxicity determination

The toxicity of fusion IT fragments was evaluated using a threshold of 0.6, with a higher score indicating increased toxicity for CD19+ B-lymphocytes. The results are summarized in Table 1 [27].

Table 1. Toxicity results

	DT380	Blincyto anti-CD19	DT380-CD19	DT390	FMC63 anti-CD19	DT390-CD19
Score	0.82	0.27	0.87	0.91	0.27	0.92
Toxicity	T	NT	T	T	NT	T

3.10. Antigenicity & Allergenicity prevision

3.10.1. Antigenicity

Models were developed using bacterial, and tumor protein datasets to predict the antigenicity of complete proteins. The antigenicity scores of fusion ITs are shown in Table 2 [28].

Table 2. Antigenicity results

	Threshold	DT380-CD19	DT390-CD19
Bacteria	0.4	0.7667	0.6622
Tumor	0.5	0.6634	0.6199

3.10.2. Allergenicity

The allergenicity of proteins can be predicted by analyzing their amino acid and dipeptide composition using methods such as support vector machine (SVM), Multiple Expectation-maximization for Motif Elicitation, motif alignment search tool, allergen representative proteins, IgE epitope mapping, or a combination of these techniques. The allergenicity outcomes of chimeric ITs are presented in Table 3 [29].

Table 3. Allergenicity results

	DT380-CD19			DT390-CD19		
Prediction method	**SVM/ amino acid**	**MAST**	**IgE epitope mapping**	**SVM/ amino acid**	**MAST**	**IgE epitope mapping**
Threshold	-0.4	-------	-------	-0.4	-------	-------

Score	0.5031	No hits found	-------	0.2045	No hits found	-------
Allergenicity	Potential allergen	Non allergen	Non allergen	Potential allergen	Non allergen	Non allergen

3.11. B lymphocyte epitopes determination

3.11.1. Linear B-cell epitopes

The linear B-cell epitopes are assigned values ranging from 0 to 1, with higher scores indicating stronger epitopes. Values ≥0.8 are considered dominant. Two dominant epitopes were identified for DT380-CD19, and four for DT390-CD19. The corresponding data can be found in Tables 5 and 6 of the supplementary information [30].

3.11.2. Conformational B-cell Epitopes

Conformational B-cell epitopes were predicted using a structure-based tool for antibody epitope prediction. Each predicted epitope is assigned a value between 0 and 1, with higher scores indicating stronger conformational epitopes [31]. DT380-CD19 had 7 surface conformational epitope scores ranging from 0.574 to 0.836, while DT390-CD19 had 4 scores ranging from 0.619 to 0.752. Additional data is provided in Tables 7 and 8 of the supplementary information.

3.12. T- lymphocyte epitopes determination

3.12.1. MHC Class-I binding regions

The HLA-A/B/C epitopes of Blincyto-antiCD19 and FMC63-antiCD19 receptor domains were found to be 21.5% and 54%, respectively [32].

3.12.2. MHC class-II binding region

The predicted output is in IC50 nM units, with lower values indicating higher affinity. Peptides with IC50 values <50 nM are considered high affinity, <500 nM intermediate affinity, and <5000 nM low affinity. Most known epitopes exhibit high or intermediate affinity. HLA-DP/DQ/DR epitopes of DT380-CD19 (Blincyto) and DT390-CD19 (FMC63) receptor domains with intermediate/high affinity were 25.00/10.00% and 70.00/23.00%, respectively [33].

3.13. Ligand-receptor Docking

Interactions between DT380-CD19, DT390-CD19, anti-CD19 scFv antibodies, anti-CD19 Fab antibodies, and anti-CD19 mAb with the two extracellular immunoglobulin-like domains of

the human CD19 antigen were evaluated. The predicted docking images of ITs are illustrated in Fig. 3 (A, B), and the corresponding data is provided in Table 4.

Additional analysis results can be found in Tables 9 and 10 in the supplementary information.

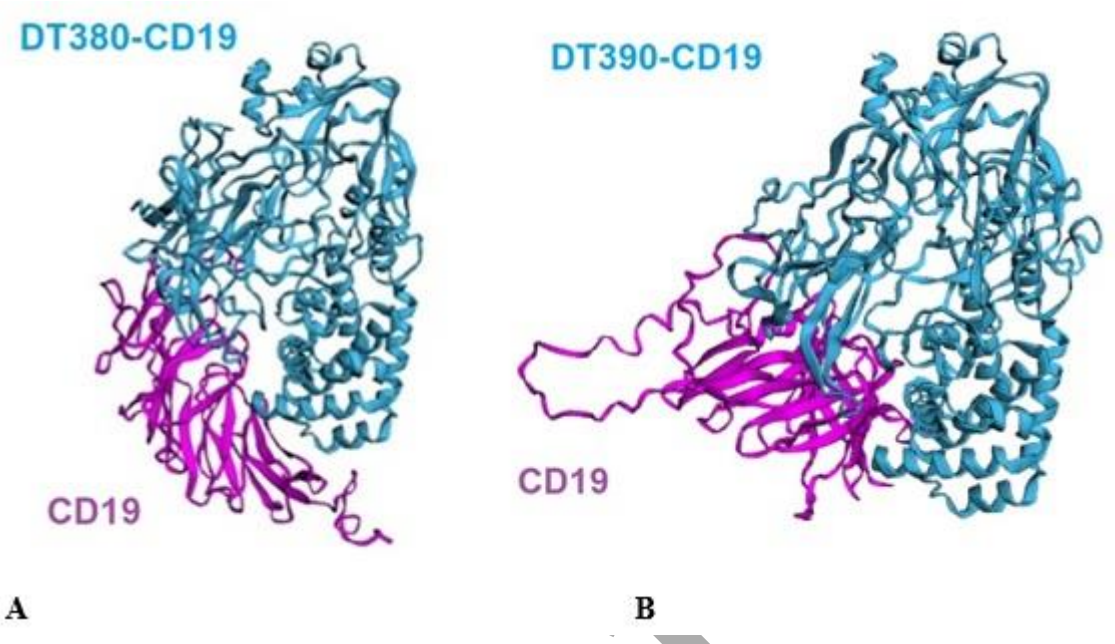

Figure 3. HawKDocK predicted ligand-receptor docking images (A, B).

Table 4. Ligand-receptor docking results

Medicines	Scores	Free energy (kcal/mol)	Affinity (ΔG)	Affinity (Kd)
DT380-CD19	- 6624.37	710.15	- 16.19	1.34×10^{-12}
Blincyto anti-CD19	- 5730.38	739.53	- 14.23	3.6×10^{-11}
DT390-CD19	- 5280.67	819.10	- 16.32	1.07×10^{-12}
FMC63 anti-CD19	- 4673.80	6748.32	-11.72	2.53×10^{-9}
Zynlonta-mAb	- 5600.97	564.14	-16.66	6.09×10^{-13}
Tafasitamab-mAb	- 5554.96	3195.46	-10.33	2.65×10^{-8}
6AL5-Fab	-------	-------	- 11.70	5×10^{-9}
SJ25C1-Fab	-------	-------	-10.00	4.3×10^{-8}
DT390-CD19 (monovalent)	-------	-------	-------	47.42×10^{-9}
DT390-CD19 (bivalent)	-------	-------	-------	4.59×10^{-9}

DT390-CD19 (foldback diabody)	-------	-------	-------	11.22×10^{-9}

3.13.1. HawKDocK score

The HawKDocK score evaluates the quality and stability of binding poses using energy terms like van der Waals, electrostatic, and desolvation potentials. Lower scores indicate superior binding poses [34].

3.13.2. Binding free energy

A lower binding free energy typically indicates a more stable and favorable interaction between the ligand and receptor proteins [34].

3.13.3. Binding affinity (delta G-binding free energy)

The ΔG value is a thermodynamic measure that indicates the spontaneity and stability of a binding interaction. A negative ΔG value indicates a favorable and spontaneous binding process, with a more negative value indicating a stronger binding affinity [34].

3.13.4. Binding affinity (Kd-dissociation constant)

The Kd value represents the equilibrium dissociation constant, indicating the concentration of ligand required to occupy half of the binding sites on the protein. A lower Kd value indicates higher binding affinity [37].

4. Discussion:

In silico studies are a cost-effective and increasingly preferred method for researchers to gain computational insights into drug evaluation and design. This study aims to focus on the latest comparative findings regarding anti-CD19 immunotherapeutics, despite the limited number of published papers.

The cytotoxic efficacy of peptide therapeutic candidates is a critical factor in clinical applications [27]. The toxicity profiles of DTx-based FDA-approved ITs such as Ontak and Elzonris are well-documented [9,10]. Our findings confirm that Blincyto, FMC63, Zynlonta, and Tafasitamab antiCD19 antibodies do not exhibit toxicity. In contrast, DT380-CD19 and DT390-CD19, which contain the DTx toxic domain, showed toxicity towards CD19+ B-lymphocyte eukaryotic cells, indicating their potential efficacy in clinical applications [14,16]. The current study provides important insights into antigenicity and allergenicity, which are crucial for predicting allergic reactions like rhinitis, asthma, eczema, and anaphylactic shock

[28,29]. In this context, the order of decreasing antigenicity and allergenicity effects is Blincyto > FMC63 > Zynlonta > Tafasitamab. Consequently, the production of anti-drug antibodies is probable, necessitating the potential use of immunosuppressive drugs during immunotherapy treatments.

Blincyto and FMC63 exhibited higher immunogenicity in both linear and conformational B-cell epitopes compared to Zynlonta and Tafasitamab. The decreased antigenicity and immunogenicity of Tafasitamab can be attributed to its humanization engineering. Ten linear B-cell epitopes were identified for DT380-CD19, with 40% located on the anti-CD19 region, 20% on the C domain (1 dominant epitope), and 40% on the T domain. Similarly, ten linear B-cell epitopes were identified for DT390-CD19, with 40% on the anti-CD19 region, 20% on the C domain (1 dominant epitope), and 40% on the T domain (3 dominant epitopes). This suggests that Blincyto's anti-CD19 has a higher linear B-cell epitope score with 1 dominant epitope compared to FMC63's anti-CD19, which lacks a dominant epitope. Overally, DT390-CD19 has a higher number of dominant linear B-cell epitopes (4 versus 2) compared to DT380-CD19, potentially leading to increased linear B cell-associated immunogenicity [30]. Regarding conformational B-cell epitopes, 7 epitopes have been identified for DT380-CD19, while DT390-CD19 has 4 epitopes. In summary, DT380-CD19 shows a higher presence of conformational B-cell epitopes compared to DT390-CD19 [31].

In general, immunogenicity varies among individuals due to differences in immunogenetics, such as diverse MHC molecules influencing antigen presentation, variations in immune cell receptors, and signaling pathways. Our study revealed that the MHC-I epitope presentation of the DT380-CD19 receptor domain was significantly lower than that of the DT390-CD19 receptor domain, suggesting that the FMC63-antiCD19 has a greater immunogenic potential compared to the Blincyto anti-CD19. Furthermore, the anti-CD19 regions of DT380-CD19 contain fewer high and intermediate affinity MHC-II epitopes for HLA DP/DQ/DR compared to the DT390-CD19 receptor domain, indicating that DT380-CD19 has lower T cell-associated immunogenicity compared to DT390-CD19 [32,33].

The current findings from docking simulations show that the HawKDock scores ranked DT380-CD19 > Blincyto anti-CD19 > Zynlonta > Tafasitamab > DT390-CD19 > and FMC63 anti-CD19 in descending order. These results indicate that DT380-CD19 has the most favorable energy score for binding to the human CD19 antigen among the FDA-approved medications tested [34].

In terms of binding free energy, the values were ranked as follows: Zynlonta > DT380-CD19 > Blincyto-antiCD19 > DT390-CD19 > Tafasitamab > FMC63-antiCD19, indicating that DT380-CD19 and Blincyto-antiCD19 may have stronger affinity and stability compared to DT390-CD19 and FMC63-antiCD19 in their interactions with the human CD19 antigen. The ranking of ΔG binding affinity with the human CD19 antigen from high to low was: Zynlonta > DT380-CD19 > DT390-CD19 > Blincyto-antiCD19 > FMC63-antiCD19 > SJ25C1-Fab > 6AL5-Fab > Tafasitamab-mAb. Furthermore, the ranking of Kd binding affinity in descending order was: Zynlonta > DT390-CD19 > DT380-CD19 > Blincyto-antiCD19 > FMC63-antiCD19 > 6AL5 > Tafasitamab > SJ25C1-Fab [34-37]. In conclusion, the current study's findings validate the positive characteristics of the DT380-CD19, such as its favorable spontaneity, stability, and robust interaction with lower concentration parameters.

Our results were consistent with a previous study that reported the binding affinities of DT390-CD19 in various formats: bivalent, single-chain foldback diabody, and monovalent, as shown in Table 4. Interestingly, the affinity of DT380-CD19 was found to be higher than that of all formats of DT390-CD19, suggesting greater specificity and stronger binding to the human CD19 receptor.

The DT380-CD19 immunotoxin (IT) showed a stronger binding affinity to CD19 compared to anti-CD19 alone, as evidenced by lower ΔG and Kd values in Table 4. This suggests that the conjugation of DT380 to anti-CD19 not only did not hinder binding but actually improved the binding affinity of the IT. Additionally, DT380-CD19 displayed acceptable antigenicity, allergenicity, and immunogenicity when compared to DT390-CD19. It also demonstrated significant docking and binding affinity to human CD19 B-cells, which could potentially reduce side effects and enhance treatment effectiveness.

5. Conclusion:

In conclusion, DT380-CD19 shows great promise as an effective immunotherapeutic agent for treating CD19+ B-cell malignancies. However, concerns about its safety and efficacy remain significant [7]. Further research is necessary to confirm and build upon the current findings regarding the properties of DT380-CD19. This includes investigating various designs for toxic and targeting domains, enhancing the production process, and conducting preclinical and clinical trials.

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Study concept and design: D.M., M.N., M.E. and M.T.; Acquisition of data: D.M., M.N. and M.E.; Analysis and interpretation of data: D.M., M.N., M.E. and M.T; Drafting of the manuscript: D.M., M.N., and M.E.; Critical revision of the manuscript for important intellectual content: D.M., M.N., M.E., and A.Y.; Administrative, technical, and material support: M.N., M.E.; Study supervision: M.N., M.E., and M.T.

Ethics:

Not applicable

Conflicts of Interest:

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Data Availability:

All data and supplementary information are available upon request from the corresponding authors.

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