

Computational simulations of antibodies targeting the T cell receptor beta constant 1 (TRBC1) receptor in leukemia treatment

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Every year, 100,000 patients are affected by T cell cancers, including T cell leukemias and lymphomas. These mature T-cell malignancies are characterized by their aggressive nature, treatment resistance, and poor prognosis. In light of this, our research takes on a significant role. Recent studies show that the T cell receptor β -chain constant region 1 (TRBC1) protein, which is abnormally proliferated on the surface of leukemia cells, is a possible target for T cell leukemia. We hypothesize that specific antibodies can effectively target and bind to the TRBC1 receptor, providing a potential therapeutic approach for treating T-cell leukemia. In the current research, we have meticulously modeled antibodies that target these receptors and could play an essential role in killing the tumor cells. Structure elucidation of the TRBC1 receptor shows that it is a single-domain negative charged receptor containing a single binding site. This binding site is crucial since antibodies binding to this site could be used to block these receptors or detect the cancer cell. We have performed thorough molecular docking (using HDOCK software) of 10 antibodies on the TRBC1 receptor to identify the best binding antibody. The docked structures were screened based on visual inspection, the binding energy formed, and the number of hydrogen bonds. This comprehensive analysis showed that antibody 1A5F had the most potent binding, making it the best candidate for further review. The selected antibody, identified through a rigorous research process, may provide an appropriate candidate for targeting TRBC1+ cancer cells and lay the groundwork for a more hopeful future in leukemia treatment.

1. INTRODUCTION

Leukemia is a type of cancer that affects the bone marrow, resulting in excessive atypical leukocytes or white blood cells (1). Leukemia is further classified by its speed, where the name is preceded by acute or chronic, and the specific group of blood cells it attacks, where the name is preceded by lymphatic or myeloid (1). Lymphoma is cancer of the lymph nodes and lymphatic tissues and can be characterized by the presence of Reed-Sternberg cells, lymphocytes, a white blood cell type with more than one nucleus (2). Lymphoma with Reed-Sternberg cells is Hodgkin's lymphoma; without it is non-Hodgkin's lymphoma (2). These cancers differ in their behavior and treatment (3). Treatments for lymphoma and leukemia may consist of chemotherapy, radiation therapy, stem cell transplants, targeted therapy and the treatment depends on the specific cancer and its progression (4).

T cells are white blood cells essential to immune function and protect the body from harm by identifying foreign particles or antigens (5). TCRs or T cell receptors are molecules located on the surface of T cells, which are requisite to antigen iden-

tification (6). TCRs are made up of two protein chains, most commonly alpha (α) and beta (β). They may also be known as gamma and delta in an infrequent form. Each chain has a variable and constant region. The variable region or the region responsible for antigen recognition is further composed of complementarity-determining regions (CDR). CDR3 is the most variable and is crucial to antigen bonding. The constant region is responsible for tethering the TCR to the T cell membrane. The transmembrane region connects the TCR to the cell's surface and uses a cytoplasmic tail to interact with signaling molecules. The CD3 complex contains many signaling molecules and transmits signals through TCR when antigens are recognized. This may lead to a cell cascade that may result in immune response, cell proliferation, or differentiation (7).

Chimeric Antigen Receptor T-cell therapy, better known as CAR T-cell therapy, is an individualized immunotherapy used to treat cancers, particularly lymphoma and leukemia (8). The procedure begins by removing the patient's T-cells and sending them to a laboratory to be genetically modified so that they may have surface proteins that can bind to specific proteins on a

cancer cell. These are chimeric antigen receptors. These altered T cells can kill cancer cells once placed back into the patient's body. CAR T-cell therapy has provided phenomenal results in some but has proven failure in others(8). It also comes with various harmful side effects, including cytokine release syndrome and neurological toxicity, which is why it is only given to those with very advanced forms of cancer (9). The Mechanism of CAR T cell is shown in Figure 1.

Molecular docking is a method that predicts and estimates the probability of interactions between ligand and receptor proteins to conclude successful binding positions and cellular energetics (9). This technique calculates the most accurate molecular orientation between the proteins (9). Scientists use it to understand further the specific properties of the binding of two molecules (9). Our research used molecular docking to simulate the binding of 15 antibodies to the TRBC1 receptor. This involved predicting the most likely binding positions and estimating the cellular energetics of these interactions. Scientists in drug development often use molecular docking to determine compounds that will produce maximal results.

The two types of surface receptors on T cells are TRBC1 and TRBC2 (10). TRBC1 and TRBC2 have relatively equivalent distribution in normal T cells; however, in malignant T cells such as T-cell large granular lymphocytic leukemia(T-LGL) and T-cell prolymphocytic leukemia(T-PLL), there is a predominant expression of one or the other cell receptor (10). For this reason, TRBC1 is predominantly targeted in T-cell cancers. TRBC1 is explicitly associated with T-cell large granular lymphocytic leukemia (T-LGL) and T-cell prolymphocytic leukemia (T-PLL) because of its greater appearance than TRBC2 present in these particular cancers, making it a better marker (11). The complex protein structure located on the surface of T cells is known as the T cell receptor or TCR. The TCR is necessary for identifying antigens situated in antigen-presenting cells or APCs (11). The TCR complex comprises two polypeptide chains known as the α and beta (β) chains responsible for recognizing antigens (11). The CD3 complex works with TCR for intracellular response. It is composed of several constituents: CD3 γ (gamma), CD3 δ (delta), CD3 ϵ (epsilon), and CD3 ζ (zeta), which is typically found as a homodimer (12). Preclinical research suggests that targeting T cell receptor β -chain constant region 1 (TRBC1) can eliminate malignant T cells while maintaining healthy immune cells. Thus, TRBC1 is a desirable target for T-cell cancer cells. However, this treatment has had unsatisfactory outcomes in clinical studies since the CAR T cells target TRBC1 depletion due to regular T cell death. To overcome this problem, Nichakawade et al. have designed a drug-bound antibody that has been developed targeting TRBC1+ cancer cells (13). We hypothesize that the antibody's antigen binding site should interact with a specific region of the TRBC1 receptor. We have performed molecular docking simulations of 15 antibodies on the TRBC1 receptor and selected the most suitable antibody. The results show that antibody 1A5F binds strongly to the TRBC1 receptor. This is a significant finding as it suggests that this antibody could be a promising candidate for targeted therapy against T-cell cancers.

2. RESULTS

In this work, we have performed molecular docking simulations to identify antibody binding to the TRBC1 receptor present on the surface of T-cell leukemia. The binding relationships between the protein and the receptor must be assessed. We accomplished this by estimating specific binding sites on the protein

surface. Structure "a" presented in Figure 2 was generated by the GraSP web server, which uses yellow and green highlights to indicate probable binding places on the receptor. It aids in visualizing the areas where proteins may interact. To verify our findings, we must compare these potential binding sites to the results of molecular docking simulations. In addition to using the GraSP server to predict protein surface binding contacts, we also used molecular simulations to develop our findings. This procedure is vital because it lets us pick antibodies that bind at the previously identified likely binding locations. Electrostatic surface potential (ESP) calculations of the receptor may be valuable to predicting possible electrostatic interactions between protein and ligand. ESP was calculated using ChimeraX software. The ESP model produced was visualized by molecule b in Figure 2. The red atoms represent negative potential, the white represents neutral, and the blue represents positive potential.

Molecular docking simulations were performed using the HDOCK software to predict the binding between the TRBC1 receptor and antibodies. We reviewed the receptor-antibody complexes shown in Figure 3 to select the most suitable antibody. Using ChimeraX, we produced images to analyze the complexes visually. This was done to find an antibody interacting with the protein's binding region predicted in Figure 2. Three antibodies were chosen using this criterion: 3G6D and 1A5F. Next, we viewed the hydrogen bonds that were formed by these antibodies, Table 1. Antibody 3G6D was eliminated from further analysis because it only formed three hydrogen bonds. Finally, we calculated the binding energy using the PRODIGY software. This analysis showed that antibody 2XQB had the most vital binding, making it the best candidate for further review. We then computed the binding interactions and energy between the two molecules to support the conclusion further. The intensity of the interaction can be measured to ensure that the antibody and receptor remain bound using the binding energy calculation. These findings support the theory that the antibody will likely remain stable when interacting with the receptor, which is vital to its possible key role in therapeutic settings.

3. DISCUSSION

Out of the many treatments for leukemia, antibody therapies have proven to be very beneficial. For one, they include an individualized treatment in which the antibody selectively binds to antigens of leukemia cells such as CD19 or CD20, which targets cancer cells while protecting healthy ones. This results in healthier results compared to therapies like chemotherapy, targeting malignant and healthy cells. In addition, this provides a better immune response that specific antibodies may elicit as an extra line of defense, such as monoclonal antibodies, which allow the immune system to identify and fight leukemic cells. Leukemia cells may get directly attached by cytotoxic chemicals with antibody-drug conjugates, combining the advantages of targeted therapy with the substantial benefits of chemotherapy with less overall damage to the body. Antibody therapies may also be used with other medical interventions, such as CAR T-cell therapy, to treat leukemia more thoroughly and improve patient outcomes and survival rates. Antibody-based immunotherapy techniques can result in longer-lasting periods of remission because they teach the immune system to keep attacking cancer cells even after treatment.

This study's main drawback is that it only uses computer

CAR T Cell Therapy: An Overview

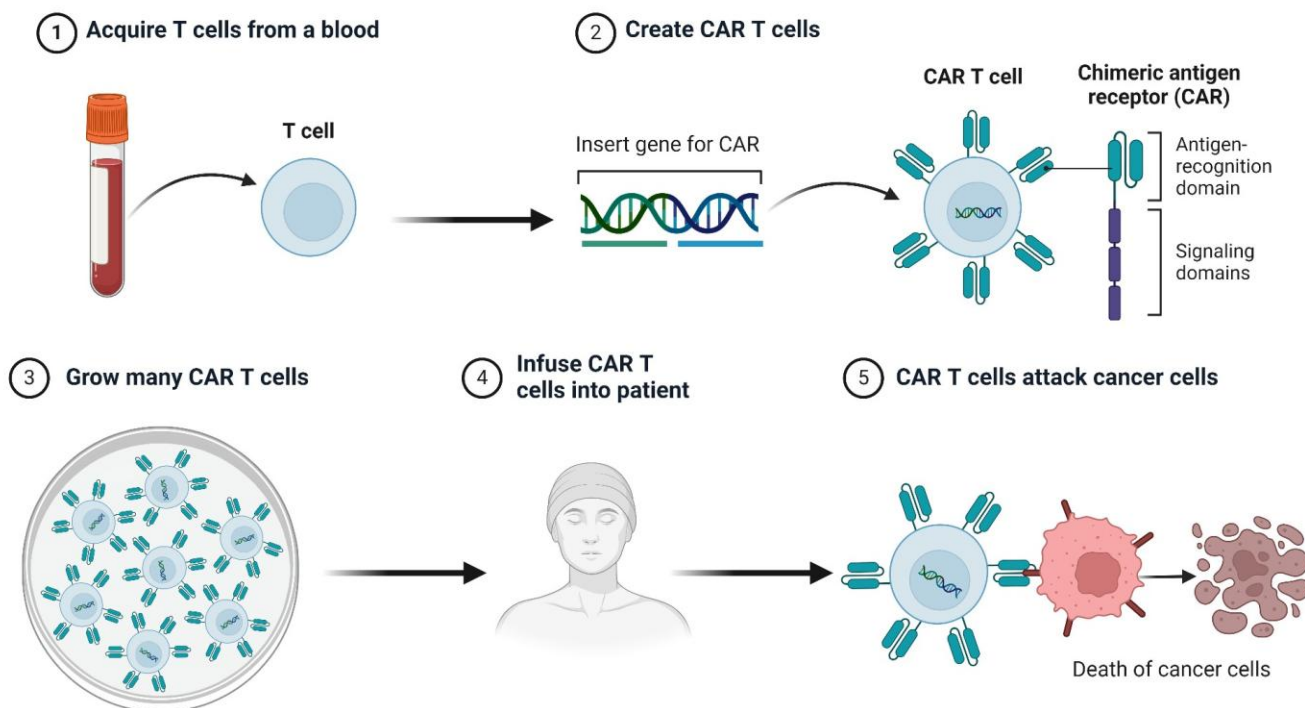


Fig. 1. Functioning of CAR T cells. The normal T cells are initially isolated from the cancer patient's body. In the next step, the CAR gene is inserted into the T cell, which results in the formation of a modified CAR T cell. These CAR T cells specifically bind to the cancer cell, resulting in cancer cell apoptosis. Created in BioRender.com [1].

Table 1. Receptor-antibody binding energy. It tells us that antibody 2vxs has the strongest interaction with the receptor.

Antibody Name	1a5f	5xrq	3hfm	1igt	1f90	3g6d	2vxs	3d85	2xqb	11k3
Binding Energy (kcal/mol)	-12.6	-11.0	-13.9	-8.2	-8.8	-10.8	-14.0	-7.6	-12.8	-11.8

simulations, which might not accurately depict the complexity of interactions within the biological microenvironment. For example, additional surface proteins, ions, and biomolecules may impact the antibody's binding affinity and specificity to the TRBC1 receptor. Furthermore, to verify the stability and effectiveness of the antibody-receptor interactions under actual biological conditions, the results must be verified through experimental techniques such as surface plasmon resonance (SPR) and isothermal calorimetry (ITC). These methods are based on theoretical models.

In the current work, we have used computational simulations to identify antibodies that can effectively target the TRBC1 receptor of specific T-cell cancers. We completed ten molecular docking simulations using HDOCK software to predict the possible ten antibodies binding with TRBC1. The docked structures were analyzed by visual inspection of the structures, hydrogen bonding, and binding energy. In addition, PRODIGY software to predict binding energy and a graph neural network to show the

number of hydrogen bonds was used to analyze the solutions further. Using the data collected, we selected antibody 1A5F. This research may apply to developing CART-cell therapies for T-cell leukemias, lymphomas, and other drug treatments.

4. METHOD

To model the TRBC1 receptor, we obtained the amino acid sequence from the UniProt website (14). Using that amino acid sequence, we inputted it into the AlphaFold 3 server to generate the image (15). AlphaFold 3 is Google's AI model that uses amino acid sequences to predict interactions between atoms and the structure of the amino acids as a protein. Using the predicted structure, the GrASP web server was able to predict the druggable binding site, which is indicated by green and yellow highlights in Figure 2a. To produce Figure 2b, the ChimeraX molecular visualization app was used to identify the electrostatic surface potential (ESP). Red regions represent positively charged sections, white regions represent neutrally charged re-

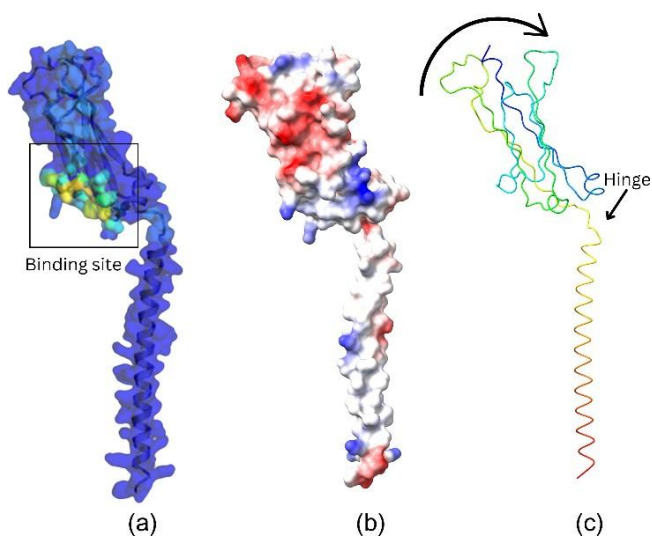


Fig. 2. Structure of TRBC1 receptor. (a) The TRBC1 receptor has a binding region as predicted by the graph neural network; (b) the electrostatic surface potential (ESP) of the receptor shows that this site is mostly negatively charged; and (c) The flexibility is computed using normal mode analysis.

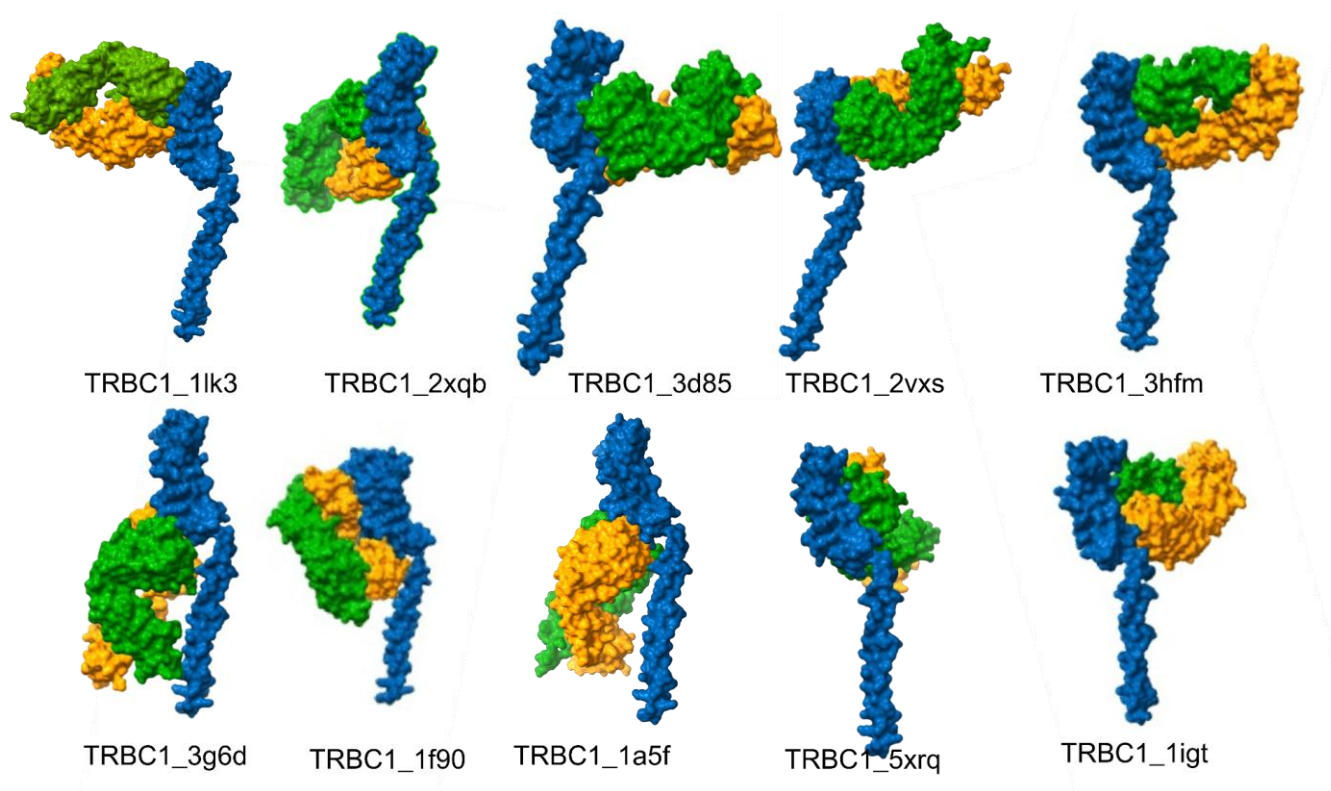


Fig. 3. TRBC1-antibody complex obtained from molecular docking simulations. Visual inspection of these structures shows that only antibodies 3G6D and 1A5F bind to the binding site predicted by the graph neural network.

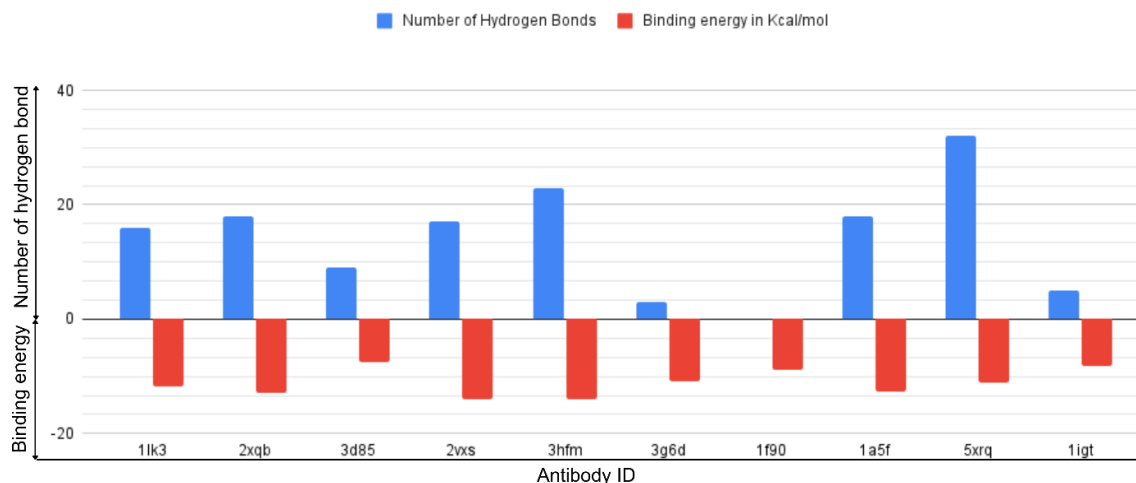


Fig. 4. Receptor-antibody number of interactions. Hydrogen bond tells the strength of interactions between the receptor and binding antibody.

gions, and blue regions represent positively charged regions. After that, ten antibodies were chosen from the protein data bank website. Using these selected antibody structures, molecular docking simulations were run using HDOCK (16). Then, ChimeraX produced the graphics. Then, Prodigy software was used to estimate the binding energy of the complex(17). Next, hydrogen bonding was calculated using an in-house Python program to compute the number of hydrogen bonds. Using these three criteria, the antibody with the most accurate binding site, the lowest binding energy, and the highest hydrogen bond number were chosen. Finally, a normal mode analysis was conducted on the TRBC1 protein to confirm flexibility and make the binding site feasible. Figure 1, used in this research was created in BioRender.com [26]

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