In Silico Identification of scFv Targeting CD22 (Siglec-2) Receptor Targeting CAR T-Cell Therapy in Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) is a fast-growing cancer of the blood and bone marrow that originates from immature lymphoid cells. The Siglec-2 (CD22) receptor is involved in cell signaling that promotes the proliferation, survival, and possibly immune evasion of ALL cells, making it a potential target for slowing disease progression. CAR T-cells (Chimeric Antigen Receptor T-cells) are immune cells that are genetically engineered to recognize and attack specific cancer cells more effectively. We hypothesize that the scFv used in this research can be attached to the surface of CAR T-cells, enabling them to target the CD22 receptor on ALL cells. In the current study, we have used computational simulation techniques to identify nanobodies that interact effectively with the CD22 receptor. The CD22 3D structure modeling was performed using AlphaFold 3, a machine learning-based technique. 9 humanized scFv structures were downloaded from the Protein Data Bank. These nanobodies were docked on the CD22 receptor using the HDOCK software. Based on the CD22-aptamer interaction analysis by PLIP software, I have identified scFv (PDB ID = 5DA0) as the most potent binding scFv to the CD22 receptor. This research can be utilized to treat ALL by using the 5DA0 scFv to target the CD22 receptor accurately, which is more prevalent in these cells. The present findings provide a foundation for the rational design of CARs targeting CD22-positive malignant cells, thereby facilitating the development of more effective and specific immunotherapies for ALL.

1. INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is a type of cancer that occurs when the bone marrow makes an excessive number of lymphocytes, which are a type of white blood cell, resulting in a shortage of red blood cells. [1] Symptoms include, and are not limited to: shortness of breath due to an enlarged thymus, pale skin, weakened immunity, bruises, increased bleeding (nosebleeds, heavy menstrual bleeding, bleeding gums), weight loss, fever, night sweats, loss of appetite, swelling in the abdomen, and spreading of symptoms to other organs. Potential methods of treatment include chemotherapy (Intrathecal and Systemic), Radiation therapy, chemotherapy with stem cell transplant, Targeted therapy, and Immunotherapy. [1] Chemotherapy tends to result in painful side effects and tends to have a higher cure rate for pediatric patients, while the cure rate for adult patients remains suboptimal. Radiation therapy can aid in eliminating the surfeit of white blood cells, however, it can also lower overall cell count, resulting in fatigue and shortness of breath (due to a low red blood cell count), bleeding or bruising (due to a low platelet count), and an increased risk of infection (due to a low white blood cell count).

The CD22 receptor is a protein receptor which is overexpressed on ALL cells. [2] The protein is partially attached to the inside of its cell, runs through the plasma membrane, and a greater portion is present external to the cell. The region near the end of its amino acid sequence is particularly more susceptible to having various sorts of interactions with other cells. This section of the receptor is able to interact with the CAR on T-cells, which can have various types of nanobodies attached to it.

Molecular Docking is a computer-based method to determine the most effective binding mode of a ligand with the three-dimensional structure of a protein. Through molecular docking, it was possible to illustrate three-dimensionally the most appropriate location for each nanobody to interact with the protein structure of the CD22 receptor. The section of the receptor which interacts the strongest with external structures was the most ideal location for the nanobodies to interact with.

CAR T cell therapy is a treatment that can eradicate advanced cancer completely for long periods of time. [3] A patient's own natural T cells, which are a part of the immune system, are used to perform this type of therapy. T cells, also known as T

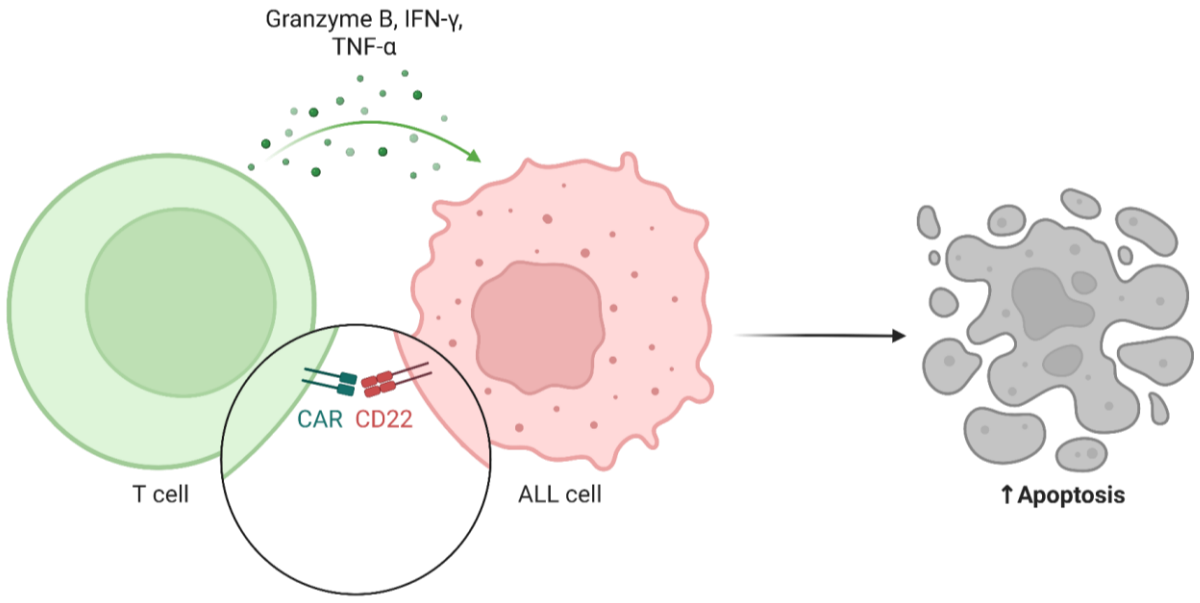

Fig. 1. This image illustrates the interaction between a CAR-T cell and the CD22 protein receptor of an ALL cell. The green cell represents a CAR-T cell, with the CAR receptor present on its surface. The red cell represents an ALL cell, which has the CD22 receptor overexpressed on its surface. Upon identification of the CD22 receptor, the CAR-T cell can release perforins that create pores on the ALL cell. The development of such pores eventually leads to apoptosis, effectively killing off the cancer cell.

lymphocytes or thymocytes, develop from stem cells in the bone marrow and usually protect the body from infections. In order to make these treatments, blood is collected from the patient and T cells are separated. These cells are then genetically engineered to produce a specific type of protein on their surface, which are known as CARs. Therefore, side effects are minimalized. [3]

2. METHODS

UniProt is a protein database, through which polypeptide amino acid sequences are stored for further analysis. [4] The CD22 protein amino acid sequence, which is overexpressed in ALL cells, was obtained from the UniProt webserver. The CD22 protein was identified as B Cell Receptor P20273 CD22_Human on the database; moreover, the database described the 847 individual amino acids. AlphaFold 3 is a Nobel Peace Prize winning technique which is used to predict protein structure. The CD22 amino acid sequence was entered into the AlphaFold 3 server, which produced the modelled structure of the CD22 protein. [5] This model was later utilized in order to determine the adequate position of various nanobodies with respect to the overexpressed CD22 protein. PROTTER is a website that provides illustrations of the CD22 protein location with respect to cancerous cells, given the protein identification name. [6] The diagram of the CD22 protein allows one to determine which segments of the amino acid sequence (847 amino acids) are intercellular, extracellular, or that pass through the plasma membrane of the cell. Furthermore, the diagram provided was interpreted in order to understand that a greater section of the CD22 receptor is located external to the cell, and at its extremity, the receptor is most prone to accepting signals, such as the various nanobodies that were tested. The signal is likely unable to pass through the cell membrane, therefore, the CD22 receptor allows for an external ligand to transmit its message to the interior of the cancerous cell. PrankWeb is a machine learning-based method which is able to predict ligand binding sites from protein structure. [7] Through this, the binding site for each of the nanobodies on the CD22 receptor protein were identified. The HDOCK web server was used for the molecular docking of the CD22 Receptor and the nanobodies as ligands. [8] The molecular docking pro-

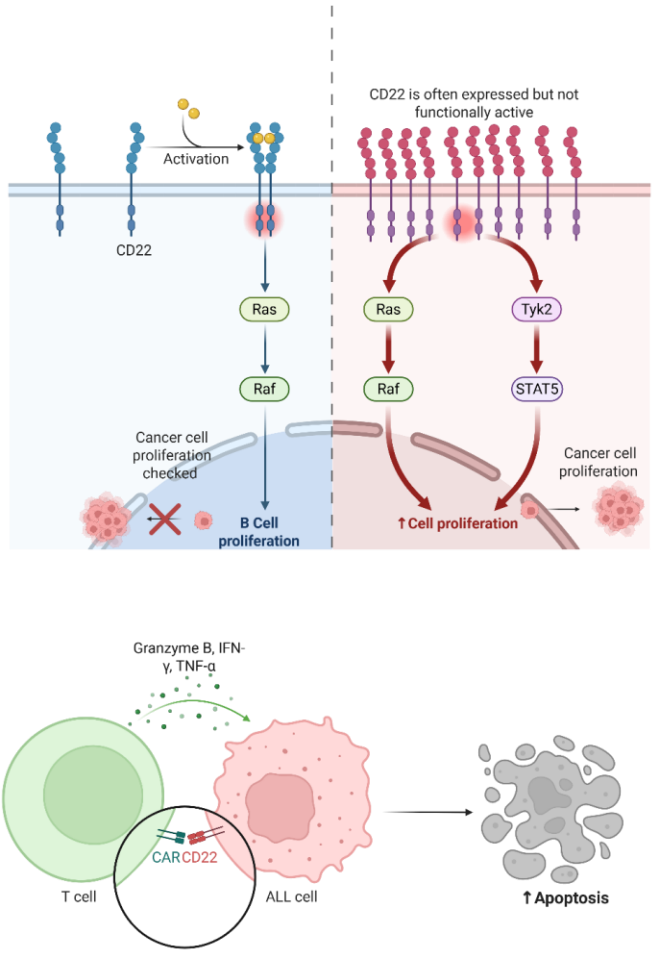

Fig. 2. Role of CD22 in cell proliferation. Active CD22 controls B-cell growth, while inactive CD22 drives Ras–Raf and Tyk2–STAT5 signaling, leading to cancer cell proliferation.

vided a visual representation of various nanobodies and their binding mode with the 3D-structure of the CD22 protein. A Protein-Ligand Interaction Profiler (PLIP) was used as a method to enumerate and analyze the chemical interactions that would occur with the CD22 protein and respective nanobodies. [9] Through analysis of hydrophobic clustering, hydrogen bonds, salt bridges, pi stacking, and pi cation interactions, it was possible to determine which nanobody most effectively pairs with the CD22 amino acid sequence.

3. RESULTS

In the current research work, we have performed computational simulations and used molecular docking to identify the most effective nanobody attached to the CAR of a CAR-T cell. The larger part of the CD22 receptor is located on the surface of ALL cancer cells and is overexpressed. Therefore, this is the area to which the nanobodies attached to the CAR on T-cells can identify and interact. Once the nanobody of the T-cell interacts with the CD22 receptor, the T-cell releases perforins, which can create pores in the affected ALL cell, eventually killing off the cell. Molecular docking simulations were performed between the CD22 receptor and various nanobodies, as shown in Figure 3.

The protein structure of the CD22 receptor is a polypeptide chain; thus, it is composed of many amino acids joined together through their carboxyl and amino termini. Additionally, each amino acid has a unique chemical structure attached to it, which is named an R-group. These chemical structures are composed of many different elements and combinations of those elements. Therefore, each structure has properties that affect how that section of the 3D structure of the amino acid chain would be shaped. There are unique properties of various sections of the protein, which allow it to interact with sections of other proteins in specific ways. Some such interactions, such as chemical bonds, are stronger than others.

4. DISCUSSION

Radiation therapy for acute lymphoblastic leukemia (ALL) involves the use of high-energy radiation to kill cancerous cells. This treatment is not a primary therapy for ALL but is applied

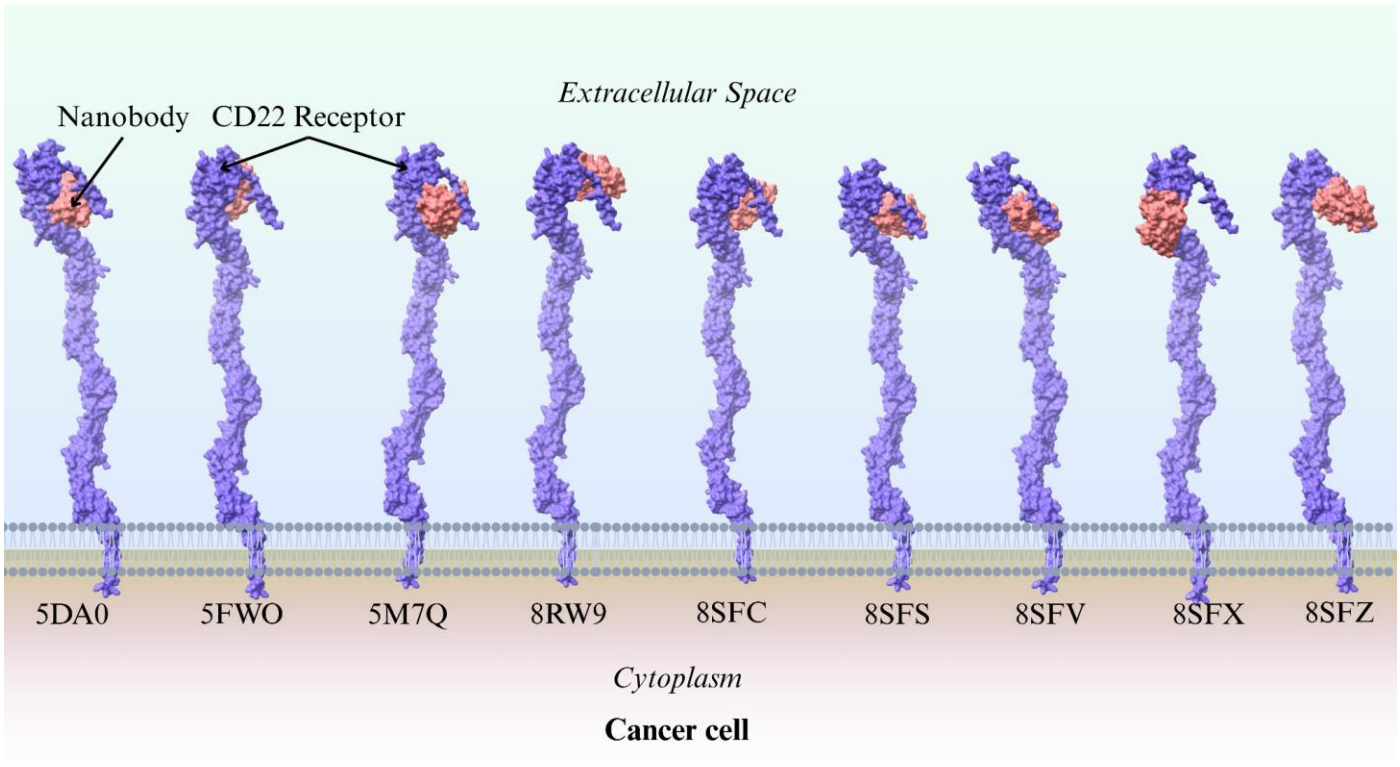

Fig. 3. The image above depicts various CD22-nanobody interactions, which were derived using molecular docking simulations on HDOCK software. There are nine types of antibodies, which were compared above in terms of their physical fit with the CD22 receptor: 5DA0, 5FWO, 5M7Q, 8RW9, 8SFC, 8SFS, 8SFV, 8XFX, 8SFZ. The structure shown in purple represents the CD22 receptor, which is attached inside the cell membrane and partially located in the extracellular space of the membrane. Each of the nanobodies, which are shown by the pink molecules, binds to its respective binding location.

in specific situations. For instance, radiation may be used if leukemia spreads to the brain, spinal fluid, or testicles (American Cancer Society, 2025). Radiation can also help reduce pain in a bone affected by leukemia when chemotherapy alone is insufficient (National Cancer Institute, 2024). In preparation for a bone marrow or peripheral blood stem cell transplant, total body irradiation may be employed to eradicate cancerous and normal bone marrow cells (Pui & Evans, 2013). Additionally, radiation can be administered if a tumor compresses the trachea and causes difficulty breathing, though this is considered a slower and less aggressive alternative compared with chemotherapy (American Cancer Society, 2025). The most common form, external beam radiation therapy (EBRT), delivers radiation from a machine to a targeted area of the body (National Cancer Institute, 2024). Before EBRT, healthcare providers carefully determine the dose, angles, and area of treatment in a process called simulation, which often involves CT or MRI imaging to ensure precision (American Cancer Society, 2025). Although radiation therapy itself is painless and relatively quick, preparation requires detailed imaging and planning. The side effects of radiation vary depending on the site of treatment. Common effects include fatigue, localized hair loss, skin irritation (burning or peeling), nausea, vomiting, diarrhea, mouth sores, difficulty swallowing, and headaches (Mayo Clinic, 2024). Radiation also affects bone marrow, lowering blood counts: reduced red blood cells may cause fatigue and shortness of breath; reduced platelets increase bruising and bleeding; and reduced white blood cells heighten infection risk (Pui & Evans, 2013).

Chemotherapy involves three phases: induction, which is a comprehensive treatment that typically lasts one month (Cancer Research UK, n.d.); consolidation, which intensifies the previous treatment and lasts for a few months (National Cancer Institute, 2024); and maintenance—also known as post-consolidation—which is a less intensive treatment that generally continues for about two years (American Cancer Society, 2025). During the first two intensive phases, patients may suffer from more serious side effects. However, chemotherapy intensity may vary depending on the subtype of acute lymphoblastic leukemia (ALL) with which the patient was diagnosed. Typically, for ALL, the cancer can spread to the brain and spinal cord, which is extremely dangerous. To analyze this spread, a spinal puncture—or spinal tap—may be performed to detect leukemia cells in the cerebrospinal fluid (CSF), the fluid that surrounds the brain and spinal cord. If leukemia is not initially present in this fluid at diagnosis, central nervous system (CNS) prophylaxis—treatment to prevent CNS involvement—is crucial to reduce the risk of spread (Merck Manuals, n.d.; American Cancer Society, 2025). Although the severity and location of ALL vary by patient, modern treatment often includes—or requires—chemotherapy. The average long-term remission or cure rate in adult patients remains modest: despite high response rates to induction, only about 30–40% of adults with ALL achieve long-term remission [10]. If the leukemia is refractory—meaning it does not respond to initial treatment—which occurs in approximately 10% to 20% of ALL cases, more intensive doses of chemotherapy may be used, though these are much less likely

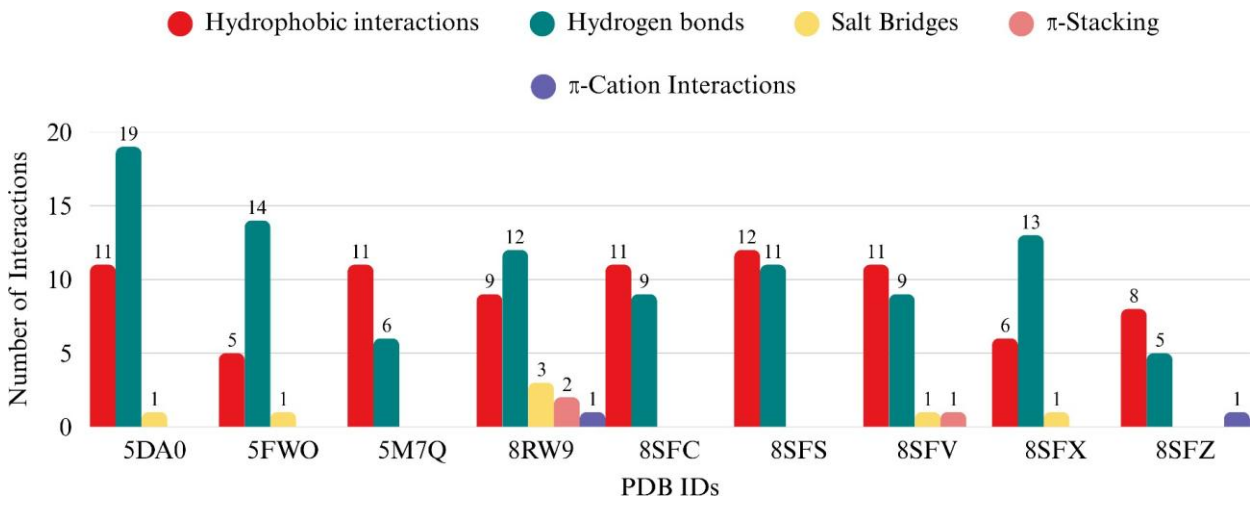

Fig. 4. The chart above describes the interactions between the CD22 receptor protein and various nanobodies. Through the use of a Protein-Ligand Interaction Profiler (PLIP), the above chart can successfully enumerate types of chemical interactions, including hydrophobic clustering, hydrogen bonds, salt bridges, pi stacking, and pi cation interactions. Out of the listed interactions, Hydrogen bonds and Salt Bridges create stronger bonds between two molecules. The 5DA0 nanobody has the most significant amount of hydrogen bonds and salt bridges combined; therefore, it bonds more strongly with the CD22 receptor. It can be confirmed that the 5DA0 nanobody most effectively pairs with the CD22 amino acid sequence; thus, nanobody 5DA0 was selected as an appropriate candidate.

to be effective (Samra, 2020). Additionally, the side effects of chemotherapy can be quite severe and vary based on dosage and duration. More common side effects include hair loss, mouth sores, loss of appetite, nausea and vomiting, and diarrhea or constipation (Leukemia & Lymphoma Society, n.d.). Chemotherapy drugs also affect healthy bone marrow cells, which can lower blood cell counts, resulting in: too few normal white blood cells, increasing infection risk; too few platelets, causing easy bruising or bleeding; and too few red blood cells, resulting in fatigue and shortness of breath (Medscape, 2024). Although medications exist to help mitigate these side effects, the treatment process can be overwhelming and exhausting for patients.

Limitations of CAR T cell therapy: Despite the numerous advantages of resorting to CAR-T cell therapy, it is relatively more expensive than other treatment methods. This is due to the intensive personalized research and examination that must take place to target ALL cells accurately to an individual's unique body. Additionally, some CAR-T cell therapies have shown toxicity. Since CAR T cell therapy is patient-specific, they are not readily available for urgent treatments.

5. CONCLUSION

In this research paper, we have used computational simulations to identify scFv candidates with strong binding affinity towards the CD22 receptor to target ALL. Among the nine scFvs analyzed, 5DA0 showed the highest number of hydrogen bonds and salt bridge interactions, suggesting its superior stability and specificity in binding. These findings highlight the potential of integrating molecular docking and interaction profiling into the rational design of CAR T-cell therapies, thereby offering a promising therapeutic avenue for targeting CD22-positive ALL cells. While further in vitro and in vivo validation will be essential, the computational results presented here lay a strong foundation for advancing more effective, targeted immunotherapies.

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